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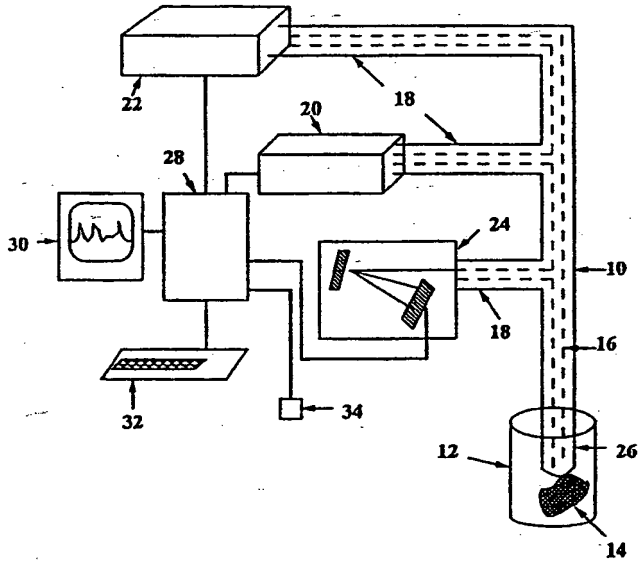

(57) Abstract

A method and apparatus for diagnosis and treatment of abnormal tissues (14) is described in which a catheter (10) with one or more optical fibers (16) is either introduced into a body cavity (12) or through an intervening body medium with the distal end positioned opposite the treatment area (14) and wherein the proximal end (18) of the catheter (10) and optical fibers (16) are coupled to a source of optical radiation (20) as well as to a method for photoelectronically sensing a particular optical characteristic of the tissue within the treatment area. Specifically, the diagnostic system and guidance is based upon sensing inelastically scattered Raman radiation in a manner which permits rapid tissue diagnosis through a computerized process of pattern recognition. Tissues which may be diagnosed encompass both normal as well as abnormal conditions including vascular occlusive diseases, *in situ* tumors or neoplastic conditions, lithiasis and calculi or any other abnormal tissue.

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OPTICAL HISTOCHEMICAL ANALYSIS, IN VIVO DETECTION AND REAL-TIME GUIDANCE FOR ABLATION OF ABNORMAL TISSUES USING A RAMAN SPECTROSCOPIC DETECTION SYSTEM

BACKGROUND OF THE INVENTION

Optical fibers, or fiberoptics as known colloquially, are finding use in a wide variety of medical applications including remote sensing and laser surgery. An optical fiber is a clad core of plastic or glass fiber in which the cladding has a lower index of refraction than the core of the fiber and as a result of its manufacture is capable of transmitting light in a tortuous path as defined by placement of the optical fiber. The term "laser" is an acronym for Light Amplification by Stimulated Emission of Radiation. As used herein, the term "laser" is meant to encompass a device which utilizes the principle of amplification of electromagnetic waves by stimulated emission of radiation to produce coherent radiation of ultraviolet (UV), visible or infrared (IR) wavelengths.

Cancer is a major cause of death in the United States and other developed nations. alone will be responsible for approximately 20% of all female cancer deaths in 1992. Diagnosis and treatment of cancer consumes billions of health care dollars annually. Efforts at early detection and treatment of malignancy (e.g., screening mammography) have the goal of reducing mortality by detecting cancer at an earlier, more treatable stage. A consequence of screening techniques, such as mammography, is the dilemma created by the detection of indeterminate findings (e.g. small breast nodules which cannot be classified further mammographically) which requires tissue sampling for histopathological identification utilizing a surgical biopsy. Procedures like fine needle aspiration biopsy (FNAB) may increase the rate of malignancy detection in non-palpable masses at surgical excision to above 15-30%; but the problem of inadequate or non-representative sampling (approximately 20%) still leaves large numbers of persons at risk of undetected disease. Improvements in the sampling accuracy of FNAB are therefore necessary to avoid false negative diagnoses and to reduce the need for excisional biopsy for benign pathological processes.

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Cardiovascular disease is the leading cause of death in the United States and most other industrialized nations. Percutaneous transluminal angioplasty, a technique based on balloon dilatation, has gained acceptance as a revascularization modality due to its less invasive nature and substantial cost savings compared with arterial bypass graft surgery.

This invention provides the surprising discovery that Raman spectroscopy, despite the presence of a blood field and the necessity to detect and ablate in real time, can be utilized clinically for the detection of abnormal tissue and ablation through body fluid for the guidance of laser surgery in vivo. Further, this invention demonstrates that the Raman spectrum of atherosclerotic plaque differs from normal arterial tissue and that this technique permits rapid diagnosis of tissues even when working through a blood field. The invention also provides that numerous neoplastic tissues (e.g. breast cancer, benign and malignant renal and hepatic tumors) or other abnormal conditions have unique Raman spectra which permits their rapid differentiation from their corresponding normal states. The invention provides adapting this method to Raman microscopy, in vivo and in vitro monitoring, through the use of an optical fiber probe. Thus, the use of Raman spectroscopy allows the accurate detection of abnormal tissues both microscopically and in vivo with fingerprinting accuracy in real time. This invention therefore solves the problems of tissue ambiguity associated with the laser-induced fluorescence techniques. Through these improvements, the invention provides a much needed means to utilize laser technology to detect and guide treatment of many disorders which heretofore were not subject to effective treatments.

SUMMARY OF THE INVENTION

The invention provides a method of diagnosing abnormal tissue in real time through a body fluid, including an intervening medium, in a subject comprising determining the Raman spectrum from the tissue in vivo or in vitro and comparing the Raman spectrum to that of normal tissue, the presence of an abnormal spectrum indicating the presence of abnormal tissue.

Also provided is an apparatus for identifying and ablating abnormal tissue in real time through a body fluid, including an intervening medium, in vivo, compromising: a sufficiently powered ablating laser, a monochromatic excitation laser, one or more fiber optic means to transmit the monochromatic energy, to collect Raman scattered energy and to transmit the energy from the ablating laser, a catheter means to house the fiber optic means, a Raman spectrometer to calibrate the collected Raman scattered energy, and a processing and activating means for analyzing the differences in Raman scattered energy to distinguish normal from abnormal tissue and activating the ablating laser, wherein the apparatus can detect and ablate the abnormal tissue in real time.

The invention further provides an apparatus for identifying abnormal tissue in real time through a body fluid, including an intervening medium, in vivo or in vitro compromising: a monochromatic excitation laser, one or more fiber optic means to transmit the monochromatic energy and to collect Raman scattered energy, a catheter means to house the fiber optic means, a Raman spectrometer to calibrate the collected Raman scattered energy, and a processing means for analyzing the differences in Raman scattered energy to distinguish normal from abnormal tissue.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a block diagram of a laser system embodying this invention.

FIG. 2 is a schematic longitudinal section of the flexible optical fiber catheter and coupling elements.

FIG. 3 is a schematic cross-sectional view of the flexible optical fiber catheter having optical fiber elements for collection of the Raman scattered signal arranged circumferentially about the distal end of the flexible conduit.

FIG. 4 is a schematic showing cross-sectional and longitudinal views of a flexible optical fiber catheter incorporating an inflatable means in the distal end of the catheter which accomplishes varying the diameter from minimum to maximum.

FIG. 5 is a schematic illustration of the operative use of a flexible optical fiber catheter wherein an inflatable means on the distal end of the catheter permits varying the diameter from minimum to maximum thereby allowing complete treatment of an obstructing lesion.

FIG. 6 is a schematic cross-sectional and longitudinal section of a flexible optical fiber catheter incorporating multiple fibers arranged circumferentially around an excitation fiber distally with a linear arrangement proximally.

FIG. 7 is a schematic of a dispersive Raman spectrometer system.

FIG. 8 is a diagram of a Raman spectrometer having input from a multiple optical fiber catheter with a rectangular array photoelectronic detector.

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FIG. 9 is a schematic illustration of the operative use of a flexible optical fiber probe for interrogating a mammographically detectable breast nodule.

FIG. 10 is a diagram of absorbance versus wavelength for whole blood.

FIG. 11 is a figure of the resonance Raman spectrum of atherosclerotic plaque obtained from atherectomy and endarterectomy

FIG. 12 shows the Raman spectra of fatty atherosclerotic plaque and normal arterial intimal surface.

FIG. 13 is a diagram of the Raman signal intensity versus distance for samples of plasma, saline and hemodiluted blood.

FIG. 14 shows the Raman signal intensity of atherosclerotic plaque as a function of sample acquisition time.

FIG. 15 shows the Raman spectra of breast fibrosis and a benign breast tumor (fibroadenoma).

FIG. 16 shows the Raman spectra of normal liver and hepatocellular carcinoma.

FIG. 17 shows the Raman spectra of normal colon and colon adenocarcinoma.

FIG. 18 shows the Raman spectra of normal kidney and renal cell carcinoma.

FIG. 19 shows the Raman spectra of three specimens of breast carcinoma.

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DETAILED DESCRIPTION OF THE INVENTION

The invention provides for an improved method and apparatus for diagnosing abnormal tissue in real time either within a subject through intervening tissues via the percutaneous placement of an optical fiber needle, within a body cavity though a body fluid via use of an optical fiber catheter, or of biopsy tissue specimens or of culture specimens studied microscopically in-vitro using Raman a spectroscopic microscope. Diagnosis is achieved in a subject comprising determining the Raman spectrum for the tissue in vivo or in vitro and comparing the Raman spectrum to that of normal tissues, compounds or organisms, the presence of an abnormal spectrum indicating the presence of abnormal tissue. More specifically, the invention provides a means to detect specific attributes of the tissue at the treatment site such that laser energy may be delivered to the treatment site for destroying abnormal tissues; as long as an abnormal condition is sensed, laser energy is delivered in pulsed fashion. When the abnormal condition has been totally destroyed, the invention provides for the recognition of this change such that laser treatment is terminated, leaving the adjacent normal tissues undamaged.

In accordance with this invention, there is provided a method of delivering monochromatic optical radiation to an area within a body to undergo treatment or diagnosis by the introduction of a flexible catheter system through an intervening body medium or into a body cavity until the distal end of the transfer conduit abuts the targeted area. Scattered optical radiation from the illuminated target area will be collected by the light transfer conduit and delivered to a means for photoelectronically sensing the inelastically scattered component of this optical radiation. Attributes of the treatment area will be detected and, by comparison with known standards, are judged normal or abnormal through use of a computer based algorithm. When laser therapy is indicated, pulses of laser energy will be delivered to the treatment area by the same flexible light transfer conduit when tissue at the treatment site has been deemed abnormal. By positioning the distal end of the light transfer conduit in abutment to the

treatment area, the abnormal condition may be selectively destroyed by repeated delivery of laser pulses of suitable wavelength and energy.

According to features of this invention, if the tissues at the treatment area display no abnormal intrinsic attributes, a means of enhancing the differences between healthy and abnormal tissues may be provided by introducing a suitable reagent, e.g. beta carotene or other Raman biochemical probe, into the body which will accumulate within the abnormal tissues, e.g., atherosclerotic plaque or neoplasm, and permit differentiation between normal and abnormal areas. Once the diseased tissues display an abnormal optical characteristic, the treatment laser energy may then be delivered selectively to the abnormal sites based upon this augmented difference between healthy and abnormal tissues.

In accordance with specific features of this invention, there is contemplated a method of detecting and destroying abnormal tissues including atherosclerotic plaque or occluding thrombus within a vascular channel or lithiasis with a urinary collecting system or biliary tree or a hyperplastic or neoplastic condition within a body medium or cavity which is accessible by endoscopic or percutaneous means. A flexible catheter system including optical fiber conduits for delivering excitation optical energy and collecting scattered optical energy can be introduced into the body cavity by appropriate means. Vascular occlusive diseases can be approached by percutaneous entry into the artery or vein. Nephrolithiasis can be approached by ureteroscopic or percutaneous access to the urinary collecting system. Bladder tumors can be accessed by cystoscopic means. Biliary lithiasis or tumors can be approached via endoscopic or percutaneous means, breast or prostate neoplasms can be approached by radiographic or ultrasonographic directed means and tumors of GI or pulmonary origin can be approached by endoscopic techniques, all of which permit use of optical fiber catheters, including needles and probes. Once access has been established within the appropriate body cavity or medium, an optical fiber catheter can be introduced and maneuvered until the distal end thereof is operatively positioned opposite the site of abnormal tissues. Monochromatic optical radiation from an

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excitation laser source can be delivered into the proximal end of the optical fiber catheter which can illuminate the treatment tissues opposite the distal end of the optical fiber catheter allowing an optical characteristic of the tissue at the treatment site to be detected. Specifically, the inelastically scattered photons can be analyzed via techniques of Raman spectroscopy which can permit identifying normal and abnormal conditions by analysis of their characteristic Raman spectra. When an abnormal condition is detected the invention permits the means by which laser pulses from a treatment laser can be periodically input to the proximal end of the optical fiber catheter and delivered to the treatment site opposite the distal end of the optical fiber catheter. The process of sensing optical properties from the treatment site and delivery of treatment pulses is alternated rapidly until the abnormal optical characteristics from the treatment site are no longer detected.

To implement the method of this invention, one or more laser systems capable of generating wavelengths suitable for the induction of Raman scattering and for the treatment of an abnormal condition is delivered via a flexible catheter containing one or more optical fibers arranged in a manner suitable for efficiently sensing the target area. A diagnostic laser source generates excitation energy for inducing Raman scattering and is connected to the proximal end of at least one optical fiber, which is positioned in any efficient manner, such as a central or multiple circumferentially arranged optical fibers. However, in a preferred embodiment of the present invention, optical fibers collecting the scattered radiation from the target site may be circumferentially arranged around the excitation fiber or fibers. A treatment laser source is coupled into the proximal end of the optical fiber catheter with this treatment energy delivered via either the same optical fibers used for collection of scattered energy or via closely adjacent optical fibers. The proximal end of the collecting optical fiber or fibers which deliver scattered optical radiation from the target site is coupled in an efficient manner into a dispersive type Raman spectrometer system. A holographic notch (interference) filter blocks Rayleigh scattered light and a diffraction grating or other dispersive optical element

within the Raman spectrometer separates the inelastically scattered photons by wavelength which are then photoelectronically transduced by a radiation detector means which converts the optical energy into electronic signals suitable for computer processing. A computerized algorithm incorporating neural network or other techniques of expert system implementation permits analysis of the Raman spectrum of the tissue at the treatment site to rapidly distinguish normal from abnormal conditions.

By arranging the circumferentially distributed optical fibers which detect the scattered optical radiation from the target site in a linear configuration at the input to the Raman spectrometer and by using cylindrical focusing optics and a rectangular matrix radiation detector, the optical characteristics from multiple sectors or areas of the target site may be simultaneously detected, processed and analyzed such that, for example, the entire circumference of a vessel can be analyzed simultaneously with each discrete optical fiber conveying information about a unique sector or segment of the vessel or region under study. Another implementation of this invention is a modification to the optical fiber catheter which provides an inflatable means in the distal end of the catheter whereby the diameter of the distal end of the optical fiber catheter may be varied from minimum to maximum diameter thereby permitting its use in various sized bodily channels. This modification also provides a means to treat vascular occlusions in conjunction with or in the absence of a treatment laser. Yet another implementation of the invention is a simplified system utilized strictly for diagnosis, and is embodied by a single excitation source, a single optical fiber catheter which may be utilized as a percutaneous optical fiber needle or probe in conjunction with a Raman spectrometer for remote optical histochemical tissue sampling and analysis. Such a system utilizes the same principles of detecting abnormal characteristics from the treatment site but does not provide for a means of treating the abnormal condition once detected.

To accomplish these means, the invention provides an apparatus for identifying abnormal tissue in real time through a body fluid,

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including an intervening body medium, in a subject comprising determining the Raman spectrum for the tissue in vivo or of a culture specimen or biopsy specimen in vitro and comparing the Raman spectrum to that of known normal tissue, the presence of an abnormal spectrum indicating the presence of abnormal tissue. Also provided is an apparatus for identifying abnormal tissue in real time through a body fluid, including an intervening medium, in vivo comprising: a monochromatic excitation laser, one or more fiber optic means to transmit the monochromatic energy and to collect Raman scattered energy, a catheter means to house the fiber optic means, a Raman spectrometer to calibrate the collected Raman scattered energy, and a processing means for analyzing differences in Raman energy to distinguish normal from abnormal tissue.

EXAMPLES

The invention will now be described utilizing the examples of destruction of a vascular occlusive narrowing as diagrammed in FIG. 1, and of percutaneous diagnosis of a breast mass, FIG. 9, which examples are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art. In FIG. 1, once vascular access has been established, a laser catheter 10 is inserted into the vascular tree 12 to the site of an obstructing lesion 14 using techniques known in the field of vascular interventional radiology. This laser catheter 10 houses within in it one or more optical fibers 16 that may be coupled at their proximal ends 18 to a source of laser energy (either diagnostic 20 and/or treatment 22) as well as to a dispersive Raman spectrometer 24. The distal end 26 of the laser catheter 10 is positioned opposite an area of occlusive narrowing or obstructing lesion 14. A computer system 28 controls the operation of the Raman spectrometer system 24 and lasers 20 and 22 and provide both a means of viewing the measured spectra on a monitor 30 as well as a means of providing feedback through use of a keyboard 32, mouse 34 or other well-known display means (not shown).

A diagnostic laser 20 serves as the source of monochromatic excitation energy; such sources may include UV, visible, NIR or IR wavelengths as appropriate for the clinical situation and characteristics of the tissue or obstructing lesion under study. An appropriate monochromator or filter (not shown) that will provide essentially monochromatic excitation energy may be incorporated into the diagnostic laser system. In accordance with the invention, a single laser system may be used for both diagnostic and treatment source. Such a system could be represented by an erbium:YAG laser operating in the long pulse mode with a frequency multiplier for the diagnosis mode and operating in the Q switched mode with direct output for tissue ablation in the treatment mode.

As shown in FIG. 2a, the present invention includes at least one central optical fiber 16a coupled to a plug-in optical coupler 36 which permits efficient transmission of excitation energy into the central optical fiber 16a transforming the laser beam into a smaller diameter, but still collimated beam. In its simplest embodiment (FIG. 2b), a single optic fiber 16 is utilized for both sample excitation and scattered radiation collection via signals 38 and 40 respectively. In this arrangement, the optical coupler 36 also incorporates a beam splitter assembly 42 for the efficient bi-directional transmission of light energy for simultaneous excitation and collection of Raman scattered photons. A plug-in optical fiber connector 36 (FIG. 2a) may be utilized to simplify the optical fiber alignment procedure and make the laser catheter 10 easily attached or detached from the Raman diagnostic system 24 and treatment laser(s) 20 and 22.

Because atherosclerotic plaque is distributed around the vessel lumen in either concentric or eccentric fashion and because a near-contact geometry will be essential for efficient collection of Raman scattered signals, it is preferred that the fiberoptic catheter system closely match the diameter of the vessel lumen within which it is operating. To facilitate this demand, FIG. 3 illustrates one preferred catheter configuration wherein a circumferential array of optical fibers 16b are arranged around a central excitation fiber 16a for collection of the Raman scattered energy. A central lumen permits

guidewire delivery and placement of a central optical fiber 16a delivering excitation energy.

A further modification allows for a a combination balloon/fiberoptic catheter shown in FIGS. 4a and 4b. In this scheme, as shown in FIG. 5a, the catheter 10 is initially positioned to the site of vascular occlusion 14 with the balloon deflated, e.g. using over-the-guidewire guidance. The circumference of the vessel 14 is interrogated and treated using Raman guided laser ablation. As the plaque 44 is removed from the wall of the artery, the balloon catheter 10 is slowly advanced and inflated as shown in FIGS. 5b-5d and the vessel further treated until the vessel which includes the obstructing lesion 14 is restored to its initial, undiseased diameter. Alternatively, the combination balloon/fiberoptic catheter for Raman spectrometry can be utilized without the ablating laser with only the balloon effecting treatment. Further, FIGS. 6a and 6b illustrate a composite Raman fiberoptic catheter 10 which incorporates plurality signal collection fibers 16b circumferentially distributed around an excitation fiber 16a and can be used to permit the entire circumference of a vessel to be interrogated by the Raman diagnostic and treatment system. In this system as depicted in FIG. 6a, the signal collection fibers 16b are circumferentially arranged at the distal working end 26 of the optical fiber catheter 10. At the proximal end 18 of the catheter 10, this circumferential arrangement is mapped into a linear arrangement for input into the Raman spectrometer system 24. The linear arrangement permits the Raman spectrometer system 24 to resolve the target in angular sections, thus permitting an eccentric plaque to be selectively targeted and treated. The Raman diagnostic catheter need not be limited to twelve signal collection fibers as diagrammed. A fewer or greater number could be incorporated as demanded by the dimensions of the target tissue. Furthermore, a single row or multiple circumferential rows may be provided to adequately cover the desired target area.

To have a significant clinical impact, the laser diagnostic system must operate nearly in "real-time" meaning that the tissue under study must be evaluated several times each second, e.g. less

than once every 200 milliseconds, preferably less than 150 milliseconds, and most preferably less than 40 milliseconds, such that the treatment laser may be activated with equal frequency during the laser angiosurgery procedure. Such a demand requires use of a dispersive Raman spectrometer system such as that shown in FIG. 7 which is able to resolve signals of closely adjacent wavelength by using a diffraction grating 46 or other dispersive optical element. The resulting Raman spectrum is spread linearly over a finite distance and when projected upon a photoelectronic array detector 48, the optical spectrum may be converted into electronic signals suitable for computer analysis.

In such a dispersive Raman spectrometer system, as shown in FIG. 7, scattered optical energy 40 is the delivered and focused into the input of the Raman spectrometer 24. At the input to the Raman spectrometer 24, collimating optics 50 assure maximal coupling of the scattered photons 40 into the spectrometer apparatus 24. In this embodiment, a line-rejection filter 52 such as a Rayleigh-line rejection filter, a band-rejection filter or Bragg diffraction grating may be utilized to enhance the performance of the Raman spectrometer system 24 by increasing the rejection of Rayleigh scattered photons 40. Adjustable slits 54 also permit the Raman spectrometer 24 to strongly reject the excitation laser wavelength while simultaneously allowing the Stokes or anti-Stokes scattered Raman photons 40 to pass through without attenuation to the principle diffraction grating 46. Because the resolution of the spectrometer 24 is a function of diffraction order, wavelength, grating size and separation between adjacent grooves of the grating, gratings of increasing number of lines/cm may be utilized for increased resolution or ability to separate closely adjacent wavelengths. A linear or rectangular array detector 48 which has elements and optical coatings to optimize its conversion efficiency at the excitation wavelength converts the scattered photons 40 into an electronic signal suited for computer processing and signal analysis. As illustrated in FIGS. 8a and 8b, an OMA (optical multichannel analyzer), CCD (charge coupled device) or photodiode array detector may function as the photoelectronic detector.

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When multiple optical fibers 16b input scattered optical radiation from unique areas of the targeted tissues 14, a system may be arranged to individually but simultaneously transduce this information. At the input to the Raman spectrometer 24 such a linear arrangement of signal collection fibers 16b may be focused upon the principle diffraction grating 46 by using cylindrical collimating optics. The Raman scattered signals 40 are independently dispersed and projected onto a rectangular matrix array detector 48 for photoelectronic conversion. The rectangular matrix array detector 48 is composed of rows and columns of discrete elements. Each individual row of the matrix can be electronically addressed and interrogated to obtain the Raman spectrum from a discrete sector within the vessel lumen. This signal may be processed and classified as either "normal" or "abnormal" using a computerized algorithm. In such fashion, the circumference of the vessel is divided into a number of sectors equal to the number of circumferential fibers 16b in the fiberoptic catheter 10. As increased resolution is needed, the number of circumferential fibers 16b is increased accordingly. Each individual column of the matrix can be electronically addressed and interrogated and represents a Raman scattered photon of specific energy or wavenumber. Because only specific regions of the Raman spectrum contain information relating to the molecular structure of the target tissue, only discrete sections of the Raman spectrum need be analyzed. Thus, the speed with which the Raman diagnostic device operates can be optimized to permit its operation in real-time.

As an alternative example, the invention will now be described utilizing the illustrative example of spectroscopic interrogation of a mammographically detected breast nodule as diagrammed in FIG. 9. Once a breast nodule has been detected mammographically or ultrasonographically, puncture of the breast can be performed using the sharpened tip of a biopsy needle 56 or an optical fiber needle (not shown), which is then advanced to the site of the mammographically or ultrasonographically detectable nodule using techniques that are well known in the field of interventional radiology. The laser catheter 10 or optical fiber needle may then be inserted into the breast 60 to abut the site of a mammographically or

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ultrasonographically detected lesion 60a. This laser fiberoptic catheter 10 houses within in it one or more optical fibers 16 that may be coupled at their proximal ends 18 to a source of diagnostic 20 or therapeutic laser energy 22 as well as to a dispersive Raman spectrometer 24. The distal end 26 of the laser catheter 10 is positioned opposite an abnormal area of breast tissue 62 as determined mammographically for breast. A computer system 28 controls the operation of the Raman spectrometer system 24 and laser(s) 20 and 22 and provides both a means of viewing the measured spectra on a monitor 30 as well as a means of providing feedback through use of a keyboard 32, mouse 34 or other well-known display means (not shown).

A diagnostic laser 20 serves as the source of monochromatic excitation energy; such sources may include UV, visible, near-IR or IR wavelengths as appropriate for the clinical situation and characteristics of the tissue under study.

When developing such a computerized algorithm for Raman spectroscopic tissue identification, a physician-operator must have access to a number of pathological specimens of both normal and abnormal conditions. In the current examples of treating atherosclerotic arterial obstruction and breast cancers, it will suffice to have numerous specimens of normal and atherosclerotic artery, normal breast, benign and malignant breast tumors as well as other pathological conditions (e.g. silicone breast implant leakage). By acquiring and storing in computer 28 memory the Raman spectra associated with each condition, it will be discovered that there are specific locations of the Raman peaks which are characteristic, and thus diagnostic, of the related normal and diseased states. Specific Raman peaks (TABLE I) that are identified in abnormal atherosclerotic artery, normal breast tissue, a variety of breast cancers, liposarcoma, and a variety of other pathological conditions include peaks associated with specific molecular constituents which differ between the normal and diseased conditions. These peaks are shown in the figures. In general, about ten wave numbers and especially four to six wave numbers on either side of the peak define the peak for purposes of defining the pathological condition. For example

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calcification, beta carotene and cholesterol are typically identified in atherosclerotic arteries, while normal vessel is characterized by the presence of collagen and elastin. Thus, for any pathological condition, a physician-operator skilled in the field may develop an appropriate computerized algorithm to distinguish a normal condition from an abnormal condition.

Limitations exist for Raman spectrometer systems because the Raman effect is a weak one when compared to the energy of Rayleigh scattering and fluorescence. Competitive fluorescence is favored when using visible light energies for sample excitation. The efficiency and intensity of Raman scattering is favored as the excitation wavelength is shifted into the longer wavelength region. Because excitation of endogenous fluorophores in the samples is minimized, the Raman signals from the structural molecules of the sample are maximized such that the compositional makeup and major chemical constituents of tissues, e.g. water content, protein, elastin collagen, organic (e.g. lipids) and inorganic constituents (e.g. calcium hydroxyapatite) are discernible. A second virtue of operating at wavelengths in the NIR is the fact that the absorbance of blood is near minimum at these wavelengths (>700nm) as shown in FIG. 10.

A rapid computer algorithm will permit the acquired Raman signals to be analyzed and compared to an archived database of normal and abnormal tissues. For tissue diagnosis, a broad or narrow spectral region of the Raman scattered signal may be interpreted as appropriate for the tissue under study. The computer system will provide graphical and numeric information to the operator-physician who will then determine when the optical fiber probe is suitably positioned for accurate detection and effective laser therapy of the tissue under study. This Raman spectroscopic diagnostic system may also be limited to signal detection and tissue diagnosis without an associated treatment laser option.

To permit identification of tissues, it must be possible to recognize specific signatures or fingerprints from tissues and to classify or categorize these as "normal" or "abnormal". This process

is enabled by archiving a database of "normal" and "abnormal" tissues and storing the salient portions of these spectra in a database against which the computer system may then compare an unknown. As future tissues are characterized, this database may be updated permitting use of this Raman spectroscopic guidance system in a periodically updated role. As future specific clinical applications arise, tissues may be characterized spectrophotometrically using a Raman spectrometer through the analysis of inelastically scattered photons with certain unique details of the spectra allowing sample recognition.

Raman spectroscopy is useful for in vivo recognition of atherosclerotic plaque and may serve as a practical guidance modality adaptable to controlling laser treatment of tissues. Fatty and fibrous atherosclerotic plaque may be easily distinguished from normal arterial tissue (FIGS. 11,12). Atherosclerotic plaque is also detectable through saline, plasma or hemodiluted blood (FIG. 13), making this modality effective for in vivo guidance. These abnormal conditions can be detected in a brief interval of time, less than 200msec, especially less than 150 msec and preferably less than 40 msec, which is rapid enough to permit real-time feedback control of a treatment laser for laser angioplasty (FIG. 14).

The Raman characteristics for various tissues have been determined (TABLE I and in FIGS. 11-19) such that this technique may be applied to specific clinical applications. Briefly, fresh and formalin fixed specimens were obtained and stored until use. Fresh specimens were stored chilled in physiologic saline and studied in the non-preserved state either in air, saline or immersed in body fluid. Formalin fixed tissues were also studied under similar experimental conditions. The Raman spectra of tissues were collected, analyzed and tabulated as follows. The results with in vivo tissues will be identical to those of fresh, nonpreserved specimens because they have been studied in the fresh, non-preserved state. There has been no alteration in the molecular structure in these tissues as would be accompanied by tissue fixation when stored in formalin or other fixative. Because Raman spectroscopy is sensitive to the molecular

composition of samples and because the molecular structures of these tissues is not altered upon harvesting and because these samples are studied shortly after collection, no change in the character or quality of these specimens is encountered from that which is found in the in vivo condition. Changes following formalin fixation have also been quantitated and are predictable in such fashion that results from fixed tissues may be extrapolated to the reults from in vivo tissues.

By using the example of atherosclerotic occlusive vascular disease and breast cancer, use of Raman spectroscopy for sample identification and laser guidance will be illustrated. Specimens of normal artery (intimal surface) and fatty atherosclerotic plaque are illustrated in FIGS. 11 and 12 while specimens of breast fibrosis, benign breast tumor (fibroadenoma) breast carcinoma, normal liver, hepatocellular carcinoma, normal colon, colon adenocarcinoma, normal kidney and renal cell carcinoma are illustrated in FIGS. 15-19. The spectra of each tissue being recorded utilizing a real-time Raman spectrometer as described above. The computer program will search for the characteristic spectral peaks associated with specific molecular components known to exist in atherosclerotic plaque, or other normal and malignant conditions as illustrated. The computer program determines that in the examined range of Raman scattered wavelengths, that normal intima has only identifiable Raman spectral peaks associated with collagen and elastin and thus meets the definition of being a normal, nonobstructed vessel. By contrast, the sample of atherosclerotic plaque when illuminated at visible wavelengths shows prominent spectral peaks centered at approximately 1002, 1154 and 1516 wavenumbers and when NIR excitation is utilized a prominent spectral feature is noted at approximately 1440 wavenumbers. With reference to TABLE I, it is determined that these represent the peaks of beta carotene that are centered between 978-1043, 1126-1187 and 1482-1574 respectively or cholesterol centered between 1386-1500; thus by definition, tissue with this Raman spectrum is diseased due to the presence of beta carotene or cholesterol respectively for the different conditions of laser excitation. By sequentially examining the Raman spectrum from this tissue and then directing laser pulses

onto the abnormal surface to destroy it, the Raman spectrum can be utilized as a feedback means for guiding laser angiosurgery.

In the example of breast disease (FIGS. 15, 19 and Table I), Raman spectroscopic detection shows peaks principally at 1004, 1078, 1157, 1268, 1300, 1444, 1519, and 1652 wavenumbers in normal breast tissue. With reference to TABLE I, it is determined that these represent the peaks of beta carotene and lipids which define the normal state. By contrast a new peak centered at approximately 1356 wavenumbers is identified with variable intensity of the lipid and beta carotene peaks. Again, with reference to TABLE I, the presence of a peak centered between 1318-1406 wavenumbers suggests the presence of a porphyrin-type compound and by definition is diseased. Alternatively, with reference to FIG. 15, numerous Raman peaks of low intensity serve to differentiate either breast fibrosis or fibroadenoma from normal breast tissue.

Tissues already characterized include arterial tissues, both normal and atherosclerotic, as well as normal urothelium including bladder, ureter and renal collecting systems, exophytic bladder tumor, renal/ureteral calculi, cholelithiasis, breast, kidney, liver tissues and silicone. Additional tissues are easily studied and characterized by the techniques described above. Future applications of these techniques will include thrombus (both acute and organized), biliary tumors, bronchogenic tumors, head and neck tumors, breast cancer, GI malignancies, skin malignancies as well as other tumors in addition to bacteria or other infectious agents. TABLE I is a partial list of the salient features of certain tissues which will permit use of Raman spectroscopic detection and guidance in a clinical device.

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TABLE I - RAMAN SPECTROSCOPIC TISSUE CHARACTERISTICS

Tissue	Lineshape	Peak Position/ Peak Center	Intensity	Constituent
Normal artery	Broad	3075-3380	Moderate	Water
	Sharp-broad	2803-3075	Strong	Protein
	Sharp	1590-1727	Strong	Amino acids
	Sharp	1399-1510	Strong	Elastin/ collagen
	Broad	1230-1399	Weak-moderate	
Atherosclerotic plaque	Broad	3075-3380	Moderate	Water
	Sharp-broad	2803-3075	Strong	Protein
	Sharp	1590-1727	Strong	Amino acids
	Sharp	1386-1500	Strong	Cholesterol
	Sharp	1482-1574	Strong	B carotene
	Sharp	1126-1187	Strong	B carotene
	Sharp	978-1043	Moderate	B carotene
	Sharp	919-1053	Strong	Calcium phosphate
	Sharp	666-687/675	Weak	
	Sharp	1341-1420/1376	Weak	
Breast normal	Broad	570-626/601	Weak	
	Broad	699-770/730	Weak	
	Broad	810-946/870	Moderate	
	Broad	935-995/964	Weak	
	Sharp	982-1030/1004	Weak-moderate	B carotene C1
	Sharp	1020-1146/1078	Weak-moderate	Lipid L1
	Sharp-triplet	1109-1230/1157	Moderate-strong	B carotene C2
	Sharp-triplet	1109-1230/1191	Weak	B carotene C2
	Sharp-triplet	1109-1230/1214	Weak	B carotene C2
	Sharp-doublet	1220-1350/1268	Strong	Lipid L2

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Tissue	Lineshape	Peak Position/ Peak Center	Intensity	Constituent
	Sharp-doublet	1220-1350/1300	Strong	Lipid L2
	Sharp	1390-1525/1444	Moderate-strong	Lipid L3
	Sharp	1478-1570/1519	Strong	B carotene C3
	Sharp	1612-1720/1652	Moderate-strong	Lipid L4
	Sharp	1720-1772/1740	Weak	Lipid L5
Breast cancer	Sharp	982-1030/1004	Weak	B carotene C1
	Sharp	1035-1060/1049	Weak	
	Sharp	1108-1139/1125	Strong	
	Sharp	1139-1182/1157	Weak-strong	B carotene C2
	Sharp	1207-1224/1215	Weak	
	Sharp-doublet	1318-1406/1356	Strong	Porphyrin
	Sharp-doublet	1318-1406/1377	Weak	Porphyrin
	Sharp-doublet	1318-1406/1397	Weak	Porphyrin
	Sharp	1465-1488/1472	Weak	
	Sharp	1488-1562/1519	Weak-strong	B carotene C3
	Sharp	1610-1628/1617	Weak	
	Sharp	1639-1656/1650	Weak	Lipid 4
Breast fibrosis	Sharp	982-1030/1004	Moderate	B carotene C1
	Sharp	1121-1200/1157	Strong	B carotene C2
	Broad-doublet	1200-1350/1268	Weak	Lipid L2
		1220-1350/1300	Weak	Lipid L2
	Broad-sharp	1325-1410/1370	Weak	Porphyrin
	Sharp	1410-1476/1439	Moderate	Lipid L3

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Tissue	Lineshape	Peak Position/ Peak Center	Intensity	Constituent
	Sharp	1478-1570/1519	Strong	B carotene C3
	Sharp	1629-1687/1649	Moderate	Lipid L4
Kidney	Sharp	939-979/956	Weak	
	Broad	935-1035	Weak	
	Sharp	979-1035/1004	Weak-moderate	B carotene C1
	Sharp	1104-1145/1125	Strong	
	Sharp	1116-1210/1156	Weak-strong	B carotene C2
	Broad	1263-1300/1283	Weak	
	Sharp	1135-1396-1358	Strong	Porphyrin
	Sharp	1463-1486/1474	Weak	
	Sharp	1475-1558/1517	Weak-strong	B carotene C3
	Weak	1497-1564/1546	Weak	
Kidney normal	Sharp	1322-1401/1358	Strong	Porphyrin
	Sharp	1463-1486/1474	Weak	
Normal uro-thelium	Broad	3075-3380	Moderate	Water
	Sharp-broad	2803-3075	Strong	Protein
	Broad	1560-1630	Moderate	Amino acids
	Broad	1368-1496	Moderate	Connective tissues
Bladder tumor	Broad	3075-3380	Moderate	Water
	Sharp-broad	2803-3075	Strong	Protein
	Broad	1571-1729	Moderate	Amino acids
	Broad	1400-1507	Moderate	Connective tissues
	Sharp	921-979	Strong	Microscopic calcifica-tions
	Broad	536-671	Weak	
	Broad	386-486	Weak	

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Tissue	Lineshape	Peak Position/ Peak Center	Intensity	Constituent
Kidney stone	Sharp	1686-1750	Strong	Uric acid
	Sharp	1370-1474	Strong	Uric acid
	Sharp	1015-1074	Strong	Uric acid
	Sharp	593-674	Strong	Uric acid
	Sharp	200-1800	Weak-moderate	
	Sharp	919-1053	Strong	Calcium phosphate
	Sharp	1420-1520	Strong	Calcium oxalate
	Sharp	900-970	Strong	Magnesium phosphate
	Sharp	530-600	Strong	Magnesium phosphate
Subcutaneous fat	Sharp	951-993/974	Weak	
	Sharp	995-1045/1004	Moderate	B carotene C1
	Sharp-doublet	1045-1107/1066	Moderate	Lipid L1
	Sharp-doublet	1045-1107/1085	Weak-moderate	Lipid L1
	Sharp-triplet	1104-1228/1155	Moderate-strong	B carotene C2
	Sharp-triplet	1104-1228/1188	Weak	B carotene C2
	Sharp-triplet	1104-1228/1212	Weak	B carotene C2
	Sharp-doublet	1226-1362/1263	Moderate	Lipid L2
	Sharp-doublet	1226-1362/1303	Moderate	Lipid L2
	Sharp	1362-1381/1372	Weak	Porphyrin
	Sharp	1381-1500/1444	Moderate-strong	Lipid L3
	Sharp	1500-1565/1515	Moderate-strong	B carotene C3
	Sharp	1604-1699/1658	Weak-strong	Lipid L4

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Tissue	Lineshape	Peak Position/ Peak Center	Intensity	Constituent
	Sharp	1721-1768/1743	Weak	Lipid L5
Lipoma	Broad	800-931/875	Moderate	
	Sharp	995-1045/1004	Weak	B carotene C1
	Sharp	1045-1100/1085	Moderate	Lipid L1
	Sharp	1100-1137/1119	Moderate	
	Sharp	1137-1169/1155	Moderate	B carotene C2
	Sharp	1226-1362/1263	Moderate	Lipid 2
	Sharp	1226-1362/1300	Strong	Lipid 2
	Sharp	1397-1500/1444	Strong	Lipid 3
	Sharp	1500-1565/1515	Moderate	B carotene C3
	Sharp	1604-1699/1658	Strong	Lipid 4
	Sharp	1721-1768/1743	Moderate	Lipid 5
Lipo-sarcoma	Sharp	951-993/974	Weak	
	Sharp-doublet	1045-1107/1066	Weak	Lipid L1
	Sharp-doublet	1045-1107/1085	Moderate	Lipid L1
	Sharp	1107-1133/1121	Weak	
	Sharp	1144-1168/1155	Weak	B carotene C2
	Sharp-doublet	1226-1362/1263	Moderate	Lipid 2
	Sharp-doublet	1226-1362/1302	Strong	Lipid 2
	Broad	1336-1388/1368	Weak	Porphyrin
	Sharp	1381-1500/1439	Strong	Lipid L3
	Sharp	1512-1541/1525	Weak	B carotene C3
	Sharp	1609-1683/1652	Strong	Lipid L4

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What is claimed is:

1. A method of diagnosing abnormal tissue in real time through a body fluid in a subject comprising determining the Raman spectrum for the tissue in vivo and comparing the Raman spectrum to that of normal tissue, the presence of an abnormal spectrum indicating the presence of abnormal tissue.

2. The method of claim 1, further comprising adding a reagent to the subject which causes the abnormal tissue to possess a predetermined detectable Raman spectrum when excited by a given wavelength of electromagnetic radiation.

3. The method of claim 2, wherein the reagent is beta carotene and the abnormal tissue is atherosclerotic artery.

4. The method of claim 1, further comprising ablating the abnormal tissue in real time with a suitable laser.

5. The method of claim 4, wherein the real time is less than 200 milliseconds.

6. The method of claim 4, wherein the real time is less than about 150 milliseconds.

7. The method of claim 4, wherein the real time is less than about 40 milliseconds.

8. The method of claim 4, wherein energy from the ablation laser is directed through a single optical fiber.

9. The method of claim 4, wherein energy from the ablation laser is directed through a plurality of optical fibers capable of transmitting the energy either alone or in conjunction with selected other fibers.

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10. The method of claim 1, wherein the abnormal condition is selected from the group consisting of coronary or peripheral vascular occlusions, a hyperplastic or neoplastic condition, and lithiasis.

11. The method of claim 10, wherein the lithiasis is selected from the group consisting of cholelithiasis, nephrolithiasis, and urolithiasis.

12. The method of claim 10, wherein the neoplastic condition is selected from the group consisting of a breast, liver, kidney, colon, lung and prostate neoplasm.

13. The method of claim 1, wherein the comparison between the normal tissue and the test tissue is performed by a computer and the laser is signaled to ablate by a computer command under operator feedback when an abnormal condition is diagnosed.

14. The method of claim 1, wherein the abnormal tissue is an atherosclerotic plaque and the Raman spectrum contains one or more abnormal peak shown in Figure 12.

15. The method of claim 14, wherein the atherosclerotic plaque contains cholesterol deposits and the Raman spectrum contains a peak centered at about 1004, 1155 or 1515 wavenumbers.

16. The method of claim 1, wherein the abnormal tissue is a breast cancer and the Raman spectrum contains one or more abnormal peaks shown in Figure 19.

17. The method of claim 16, wherein the Raman spectrum contains a peak centered at about 1057, 1093, 1130, 1274, 1358, 1362, 1392, or 1620 wave numbers.

18. The method of claim 1, wherein the abnormal tissue is fibroadenoma and the Raman spectrum contains one or more abnormal peaks shown in Figure 15.

19. The method of claim 18, wherein the Raman spectrum contains a peak centered at about 1067, 1239, 1381, 1578, 1598 or 1629 wave numbers.

20. The method of claim 1, wherein the abnormal tissue is colon adenocarcinoma and the Raman spectrum contains one or more abnormal peaks shown in Figure 17.

21. The method of claim 20, wherein the Raman spectrum contains a peak centered at 1194, 1441, 1529, 1624 or 1652 wave numbers.

22. The method of claim 1, wherein the abnormal tissue is liver cancer and the Raman spectrum contains one or more abnormal peaks shown in Figure 16.

23. The method of claim 22, wherein the Raman spectrum contains a peak centered at 1115 or 1474 wavenumbers.

24. The method of claim 1, wherein the abnormal tissue is breast fibrosis or cyst and the Raman spectrum contains abnormal peaks shown in Figure 15.

25. A method of diagnosing a neoplastic condition in a tissue comprising detemrining the Raman spectrum for the tissue and comparing the Raman spectrum to that of a neoplastic condition, the presence of a spectrum of a neoplastic condition indicating the presence of the neoplastic condition.

26. A method of detecting the presence of a viral or bacterial microorganism in a sample from a subject comprising determining the Raman spectrum of the sample and comparing the Raman spectrum to that of the microorganism, the presence of a spectrum of a microorganism indicating the presence of the microorganism.

27. A method of detecting the presence of a compound of interest in a sample from a subject comprising determining the Raman spectrum of the sample and comparing the spectrum to that of the compound of interest,

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the presence of the spectrum of the compound of interest indicating the presence of the compound.

28. An apparatus for identifying and ablating abnormal tissue in real time through a body fluid in vivo, comprising:

an ablating laser,

a monochromatic excitation laser for generating monochromatic energy for Raman scattering,

one or more fiber optic means to transmit the monochromatic energy to identify the presence of abnormal tissue, to collect the Raman scattered energy and to transmit the energy from the ablating laser through the body fluid to treat the abnormal tissue identified,

a catheter means to house the fiber optic means,

a Raman spectrometer to calibrate the collected Raman scattered energy, and

a processing and ~~activating~~ means for analyzing differences in Raman scattered energy to distinguish normal from abnormal tissue and controllably activating the ablating laser to treat the abnormal tissue,

wherein the apparatus can detect and ablate the abnormal tissue in real time.

29. The apparatus of claim 28, wherein the fiber optic means comprises a plurality of circumferential optical fibers to transmit the monochromatic energy and collect Raman scattered energy.

30. The apparatus of claim 29, further comprising a central fiber to transmit the monochromatic energy.

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31. The apparatus of claim 29, wherein the plurality of circumferential optical fibers also can transmit energy from the ablating laser to the abnormal tissue.

32. The apparatus of claim 29, wherein the plurality of optical fibers selectively transmit ablating energy either alone or in conjunction with the remaining fibers.

33. The apparatus of claim 29, wherein the plurality of circumferential optical fibers are configured as a linear array at their proximal end connectable to the Raman spectrometer.

34. The apparatus of claim 33, wherein the linear array of detecting fibers is focused into the Raman spectrometer utilizing cylindrical optics.

35. The apparatus of claim 28, further comprising a detector for the Raman spectrometer which is comprised of a horizontally and vertically arranged rectangular matrix of detecting elements to permit individual optical fibers to be analyzed simultaneously with the resultant Raman spectrum representing a sector of the test tissue.

36. The apparatus of claim 28, wherein the excitation laser and the ablation laser utilize a different fiber optic means to convey energy.

37. The apparatus of claim 28, wherein the real time is less than 200 milliseconds.

38. The apparatus of claim 28, wherein the real time is less than about 150 milliseconds.

39. The apparatus of claim 28, wherein the real time is less than about 40 milliseconds.

40. The apparatus of claim 28, wherein the ablating laser and the excitation laser are the same laser and frequency multipliers are used to generate excitation and ablation energies of different wavelength.

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41. The apparatus of claim 28, further comprising an expandable means in the distal end of the catheter means such that the diameter of the distal end of the catheter means may be varied from minimum to maximum diameter thereby permitting its use in various size lumens or to treat a vascular occlusion.

42. An apparatus for identifying abnormal tissue in real time through body fluid in vivo comprising:

a monochromatic excitation laser for generating monochromatic energy for Raman scattering,

one or more fiber optic means to transmit the monochromatic energy to identify the presence of abnormal tissue and to collect the Raman scattered energy,

a catheter means to house the fiber optic means,

a Raman spectrometer to calibrate the collected Raman scattered energy, and

a processing means for analyzing differences in Raman scattered energy to distinguish normal from abnormal tissue.

43. The apparatus of claim 42, further comprising an expandable means in a distal end of the catheter means such that the diameter of the distal end of the catheter means may be varied from minimum to maximum diameter thereby permitting its use in various size lumens or to treat a vascular occlusion.

44. The apparatus of claim 41, further comprising a needle to house the catheter and fiber optic means.

45. An apparatus comprising:

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one or more fiber optic means to transmit the monochromatic energy to identify the presence of abnormal tissue and to collect the Raman scattered energy,

a catheter means to house the fiber optic means, and

a needle to house the catheter and fiber optic means.

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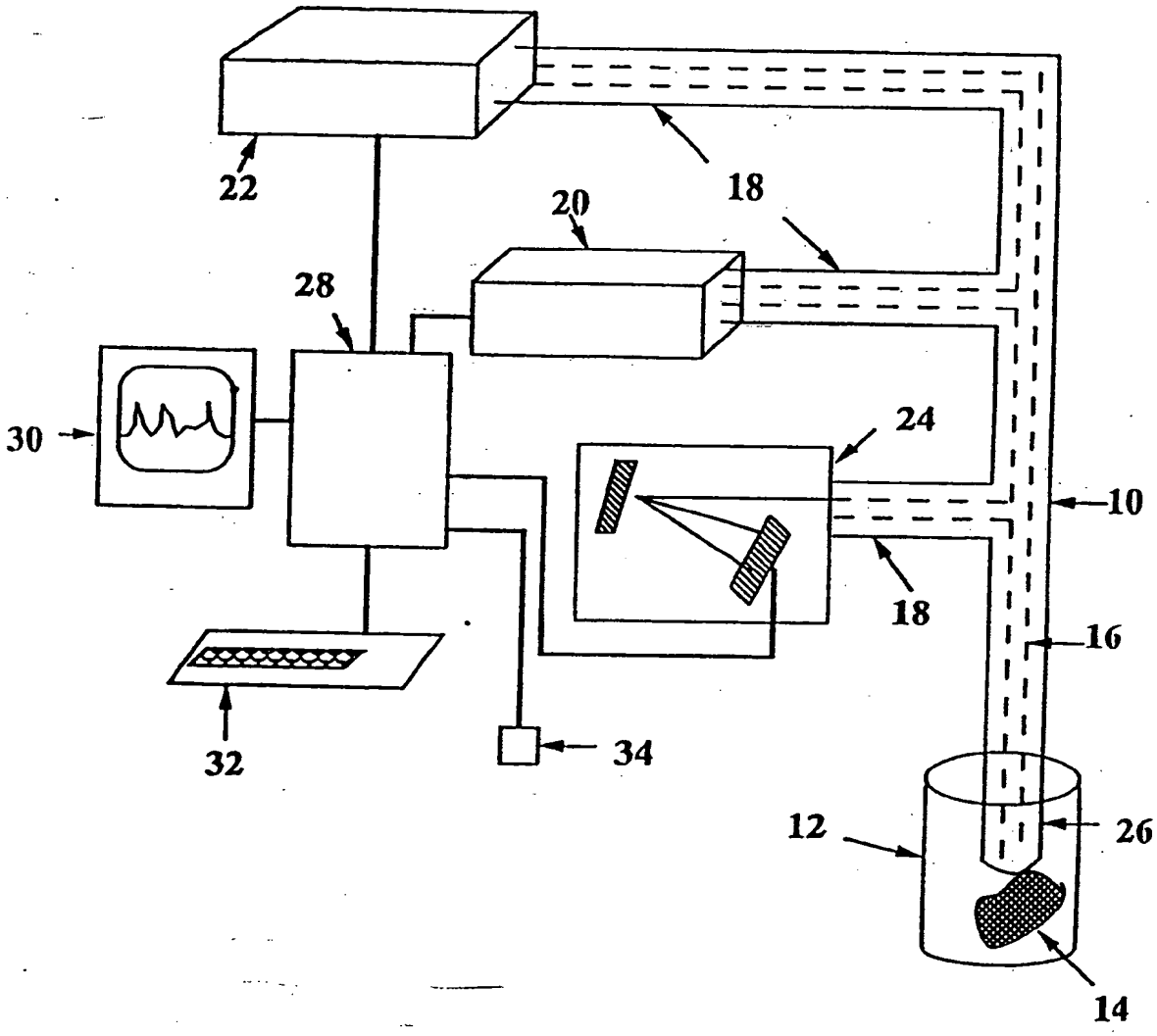

FIG. 1

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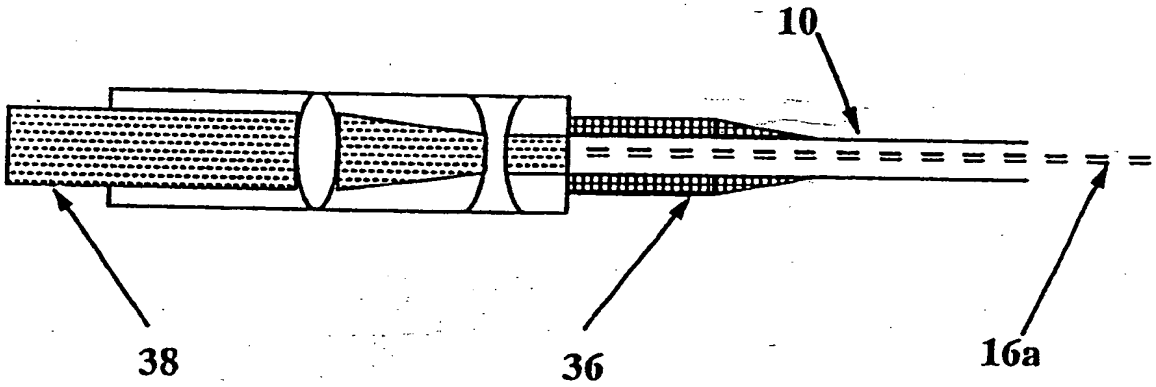

FIG. 2a

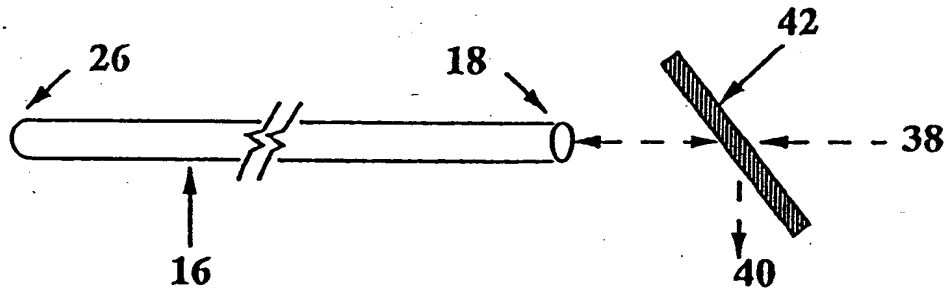

FIG. 2b

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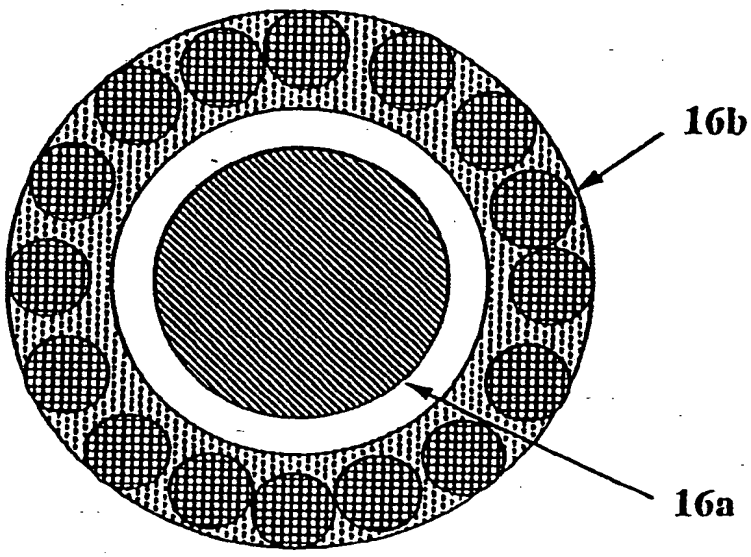

FIG. 3

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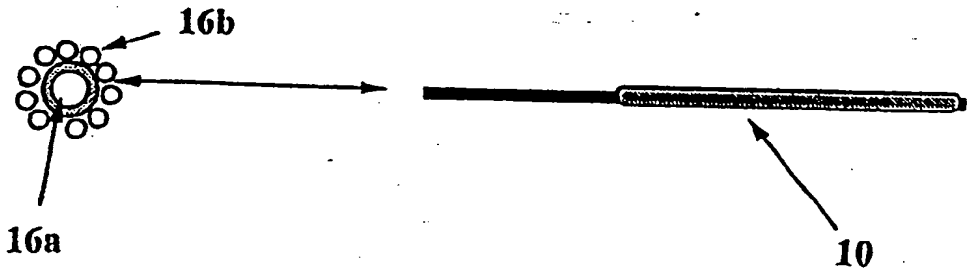

FIG. 4a

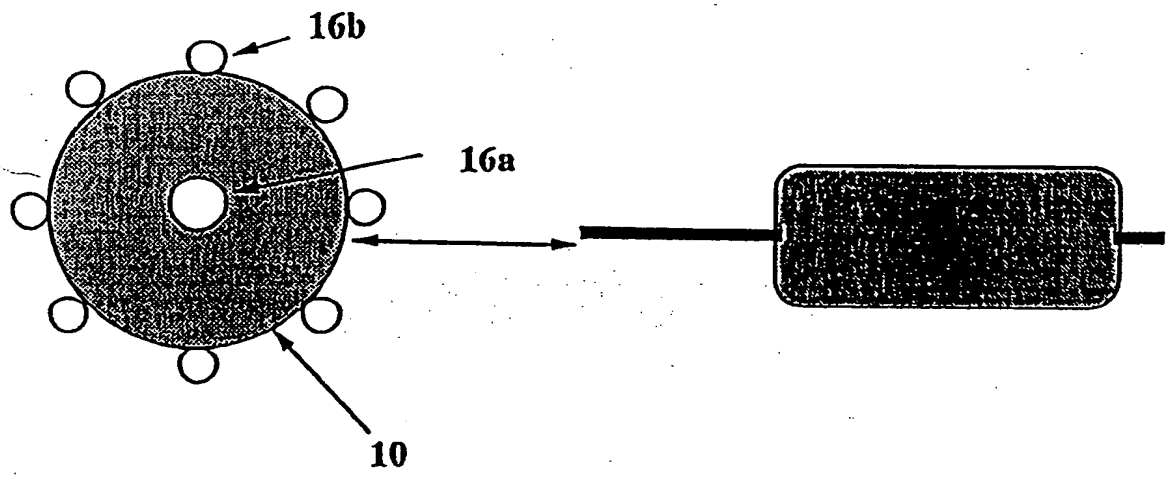

FIG. 4b

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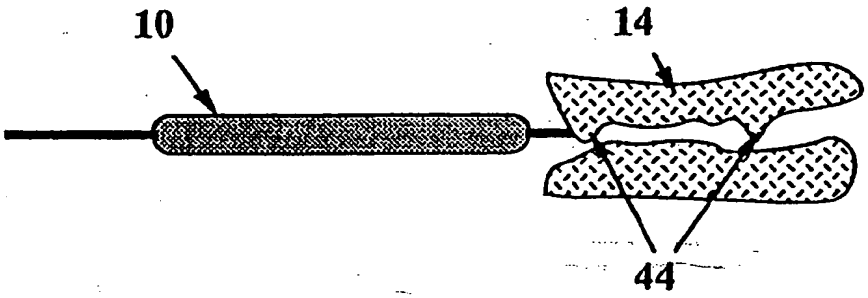

FIG. 5a

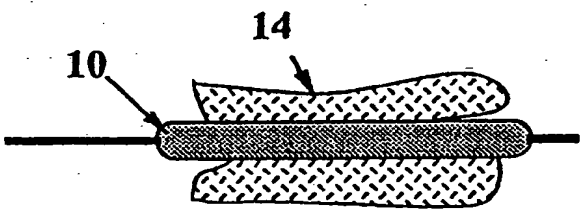

FIG. 5b

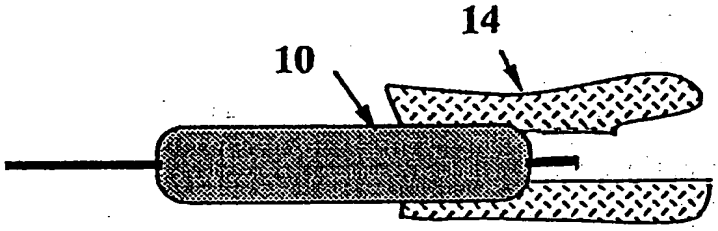

FIG. 5c

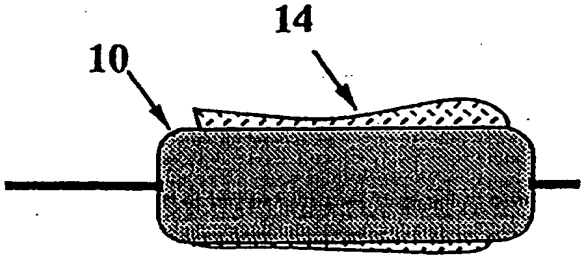

FIG. 5d

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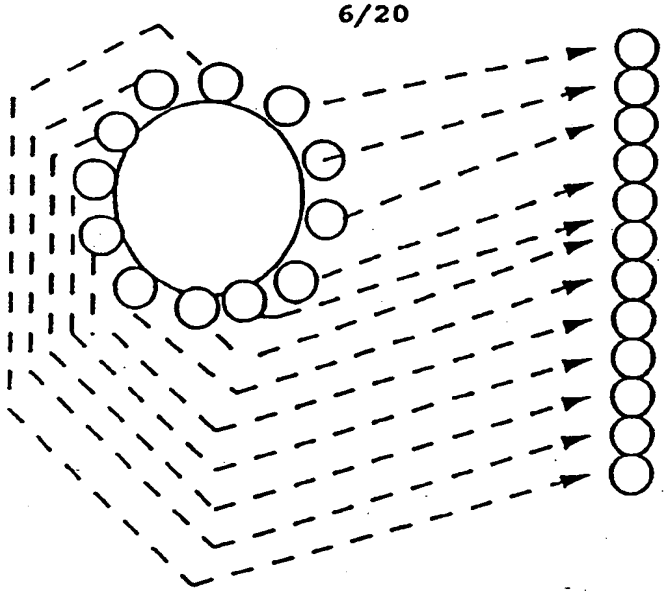

FIG. 6a

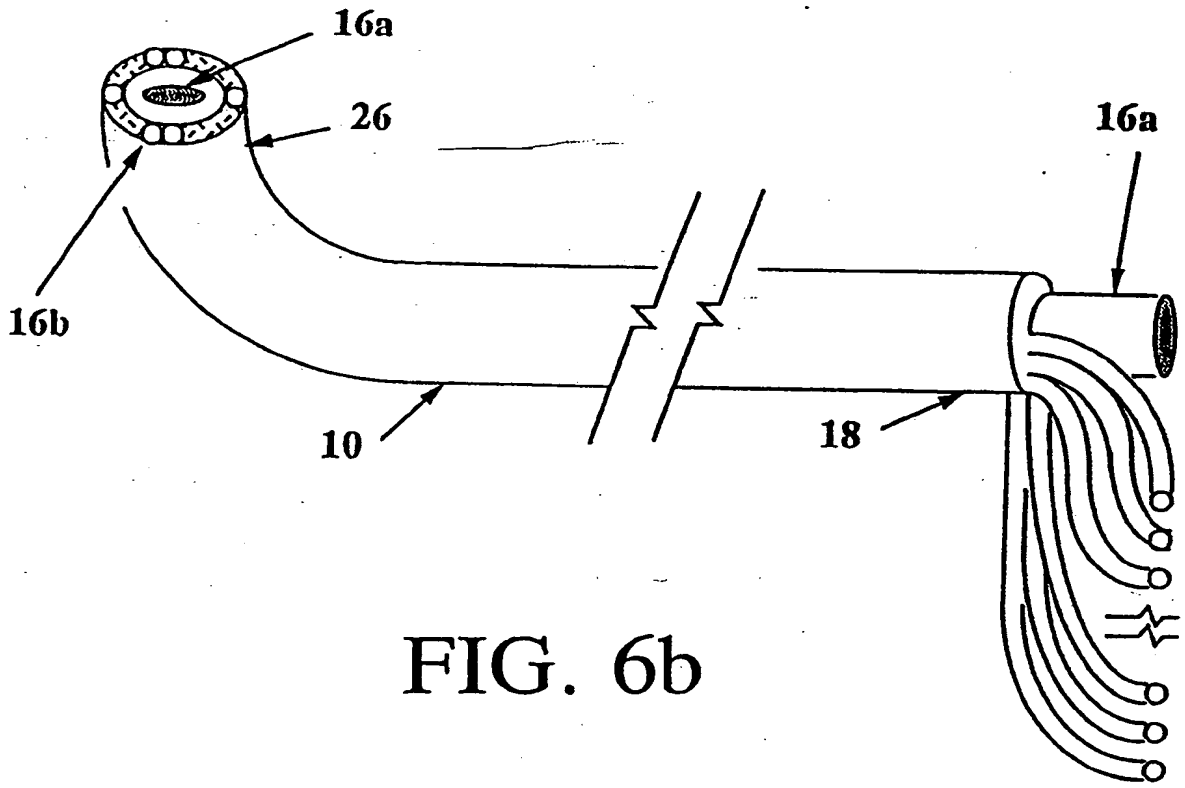

FIG. 6b

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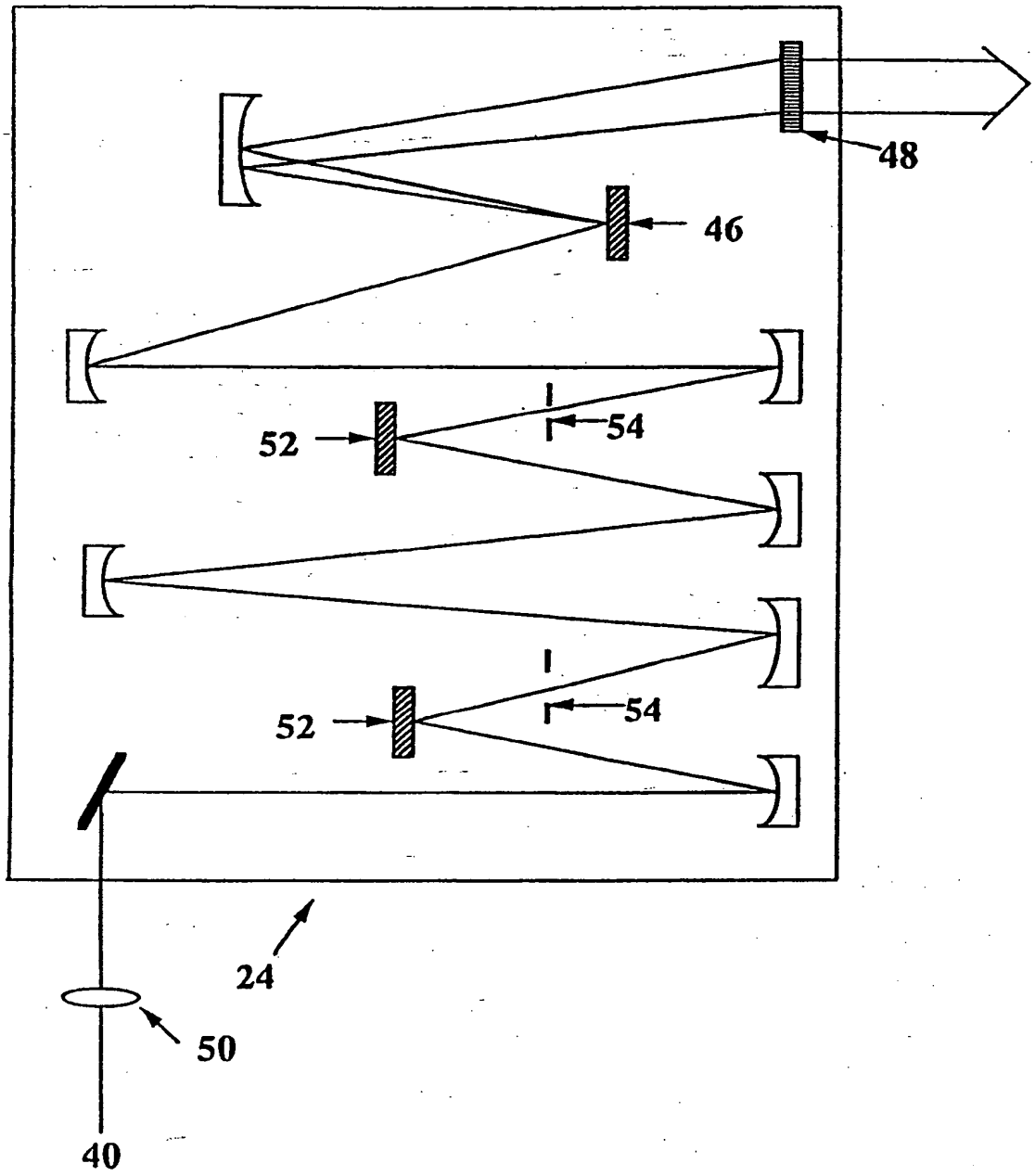

FIG. 7

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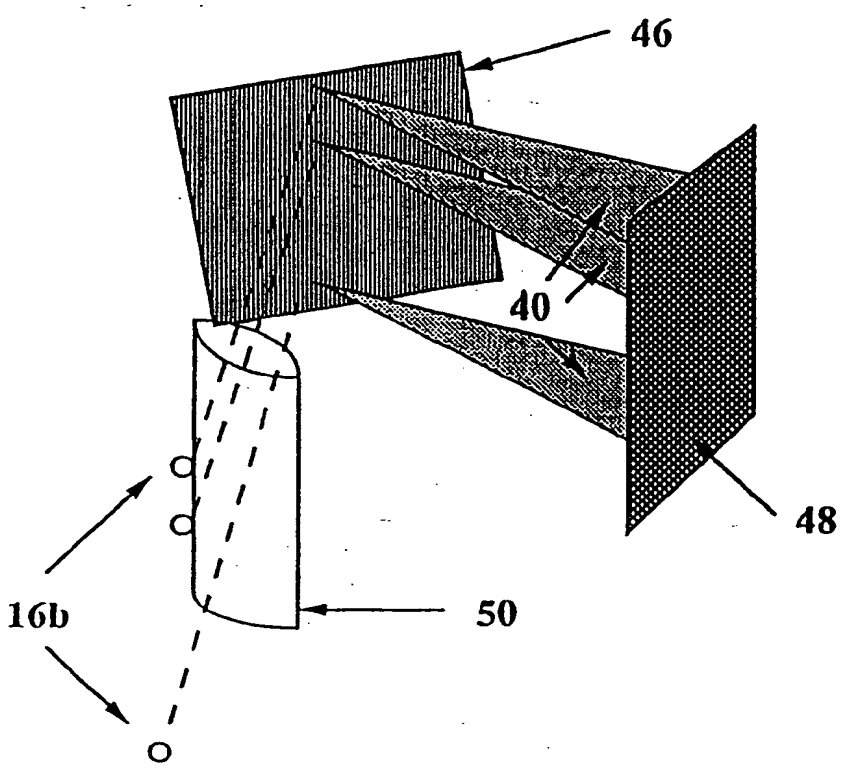

FIG. 8a

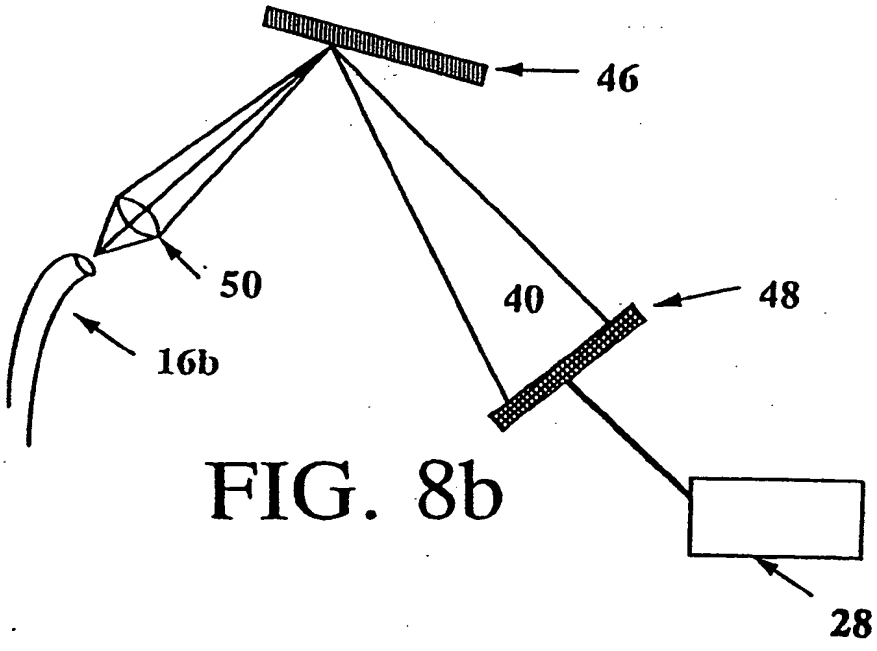

FIG. 8b

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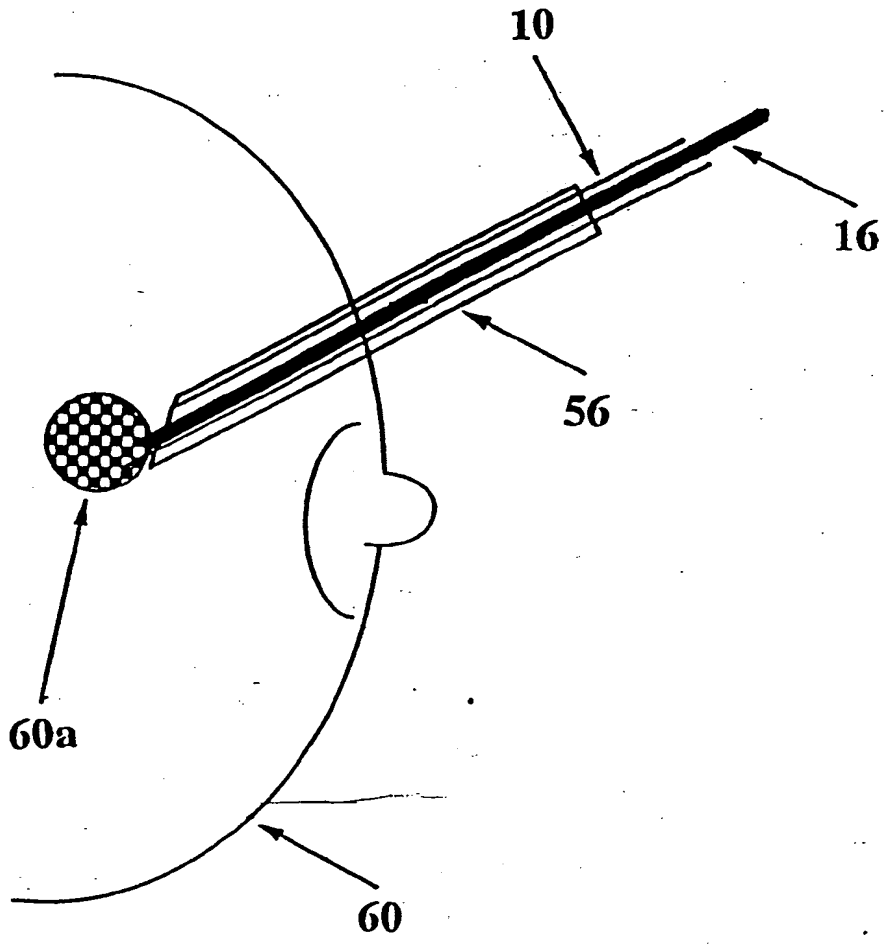

FIG.9

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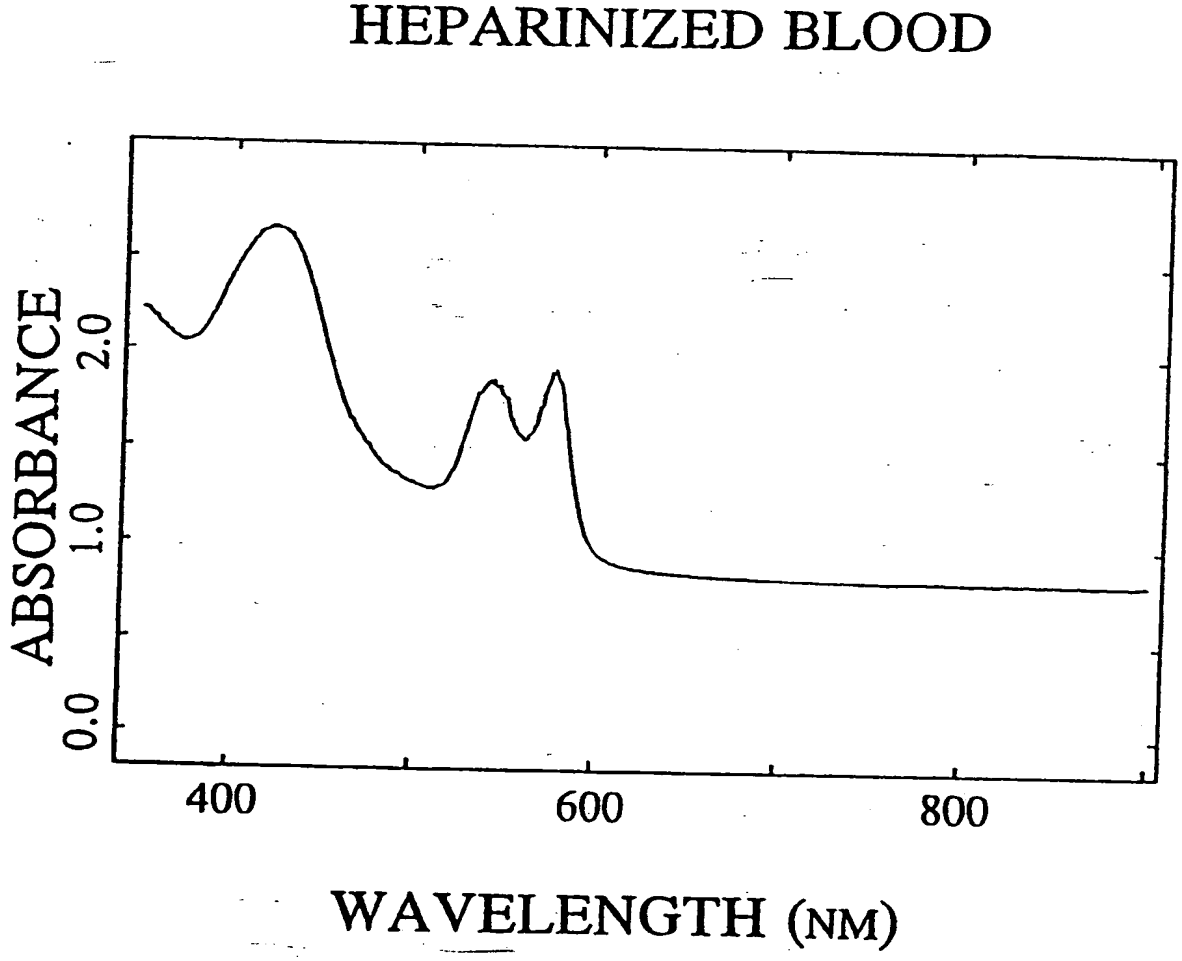

FIG. 10

BNSDOCID: <WO___9303672A1_I_>

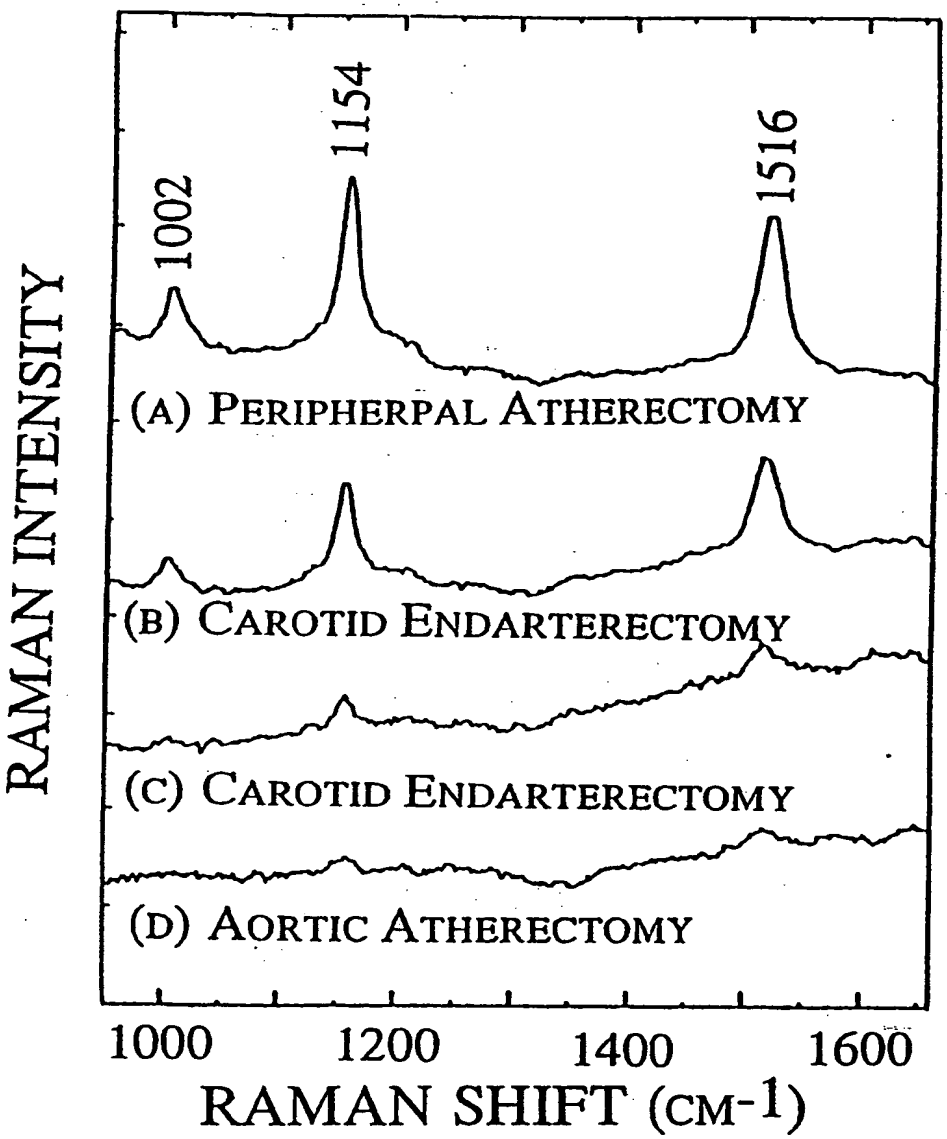

FIG. 11

BNSDOCID: <WO___9303672A1_I_>

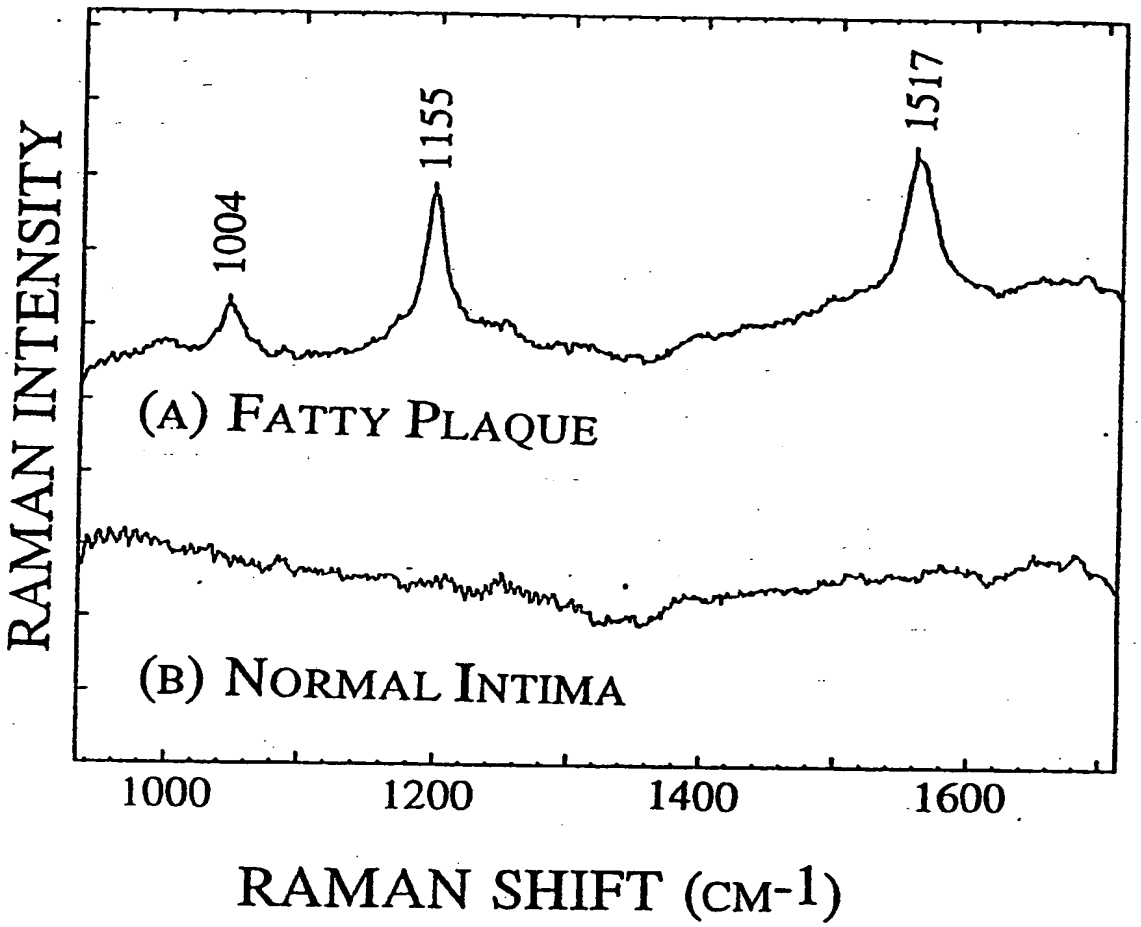

FIG. 12

BNSDOCID: <WO___9303672A1_I_>

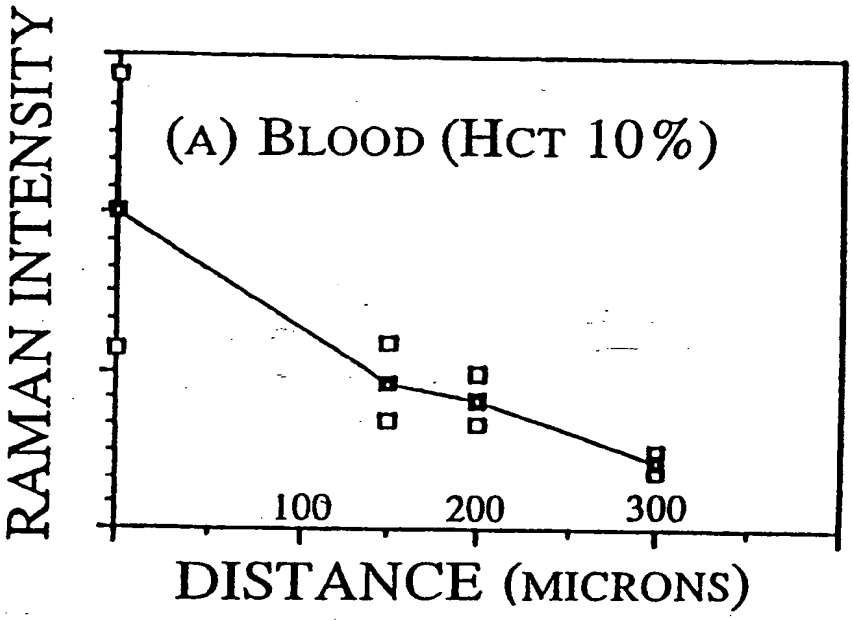

FIG. 13a

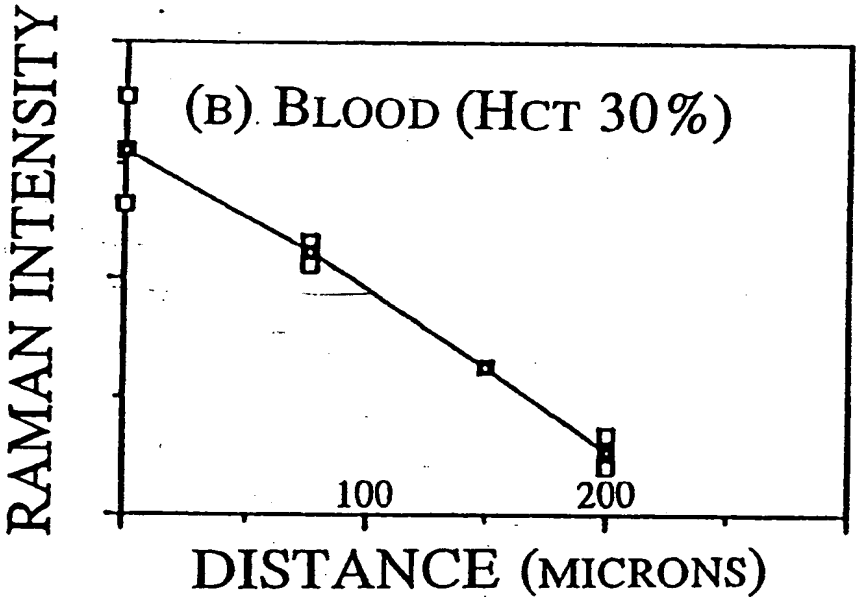

FIG. 13b

BNSDOCID: <WO___9303672A1_I_>

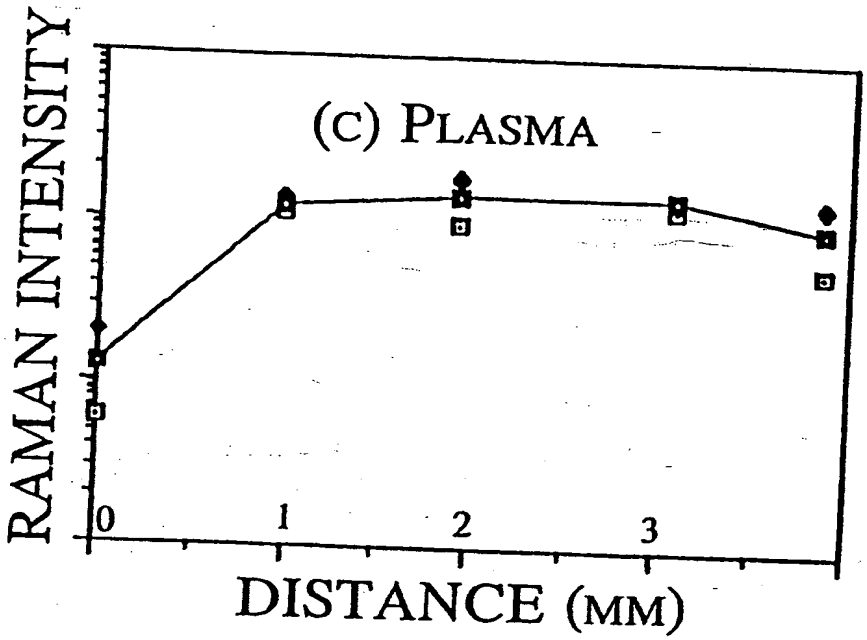

FIG. 13c

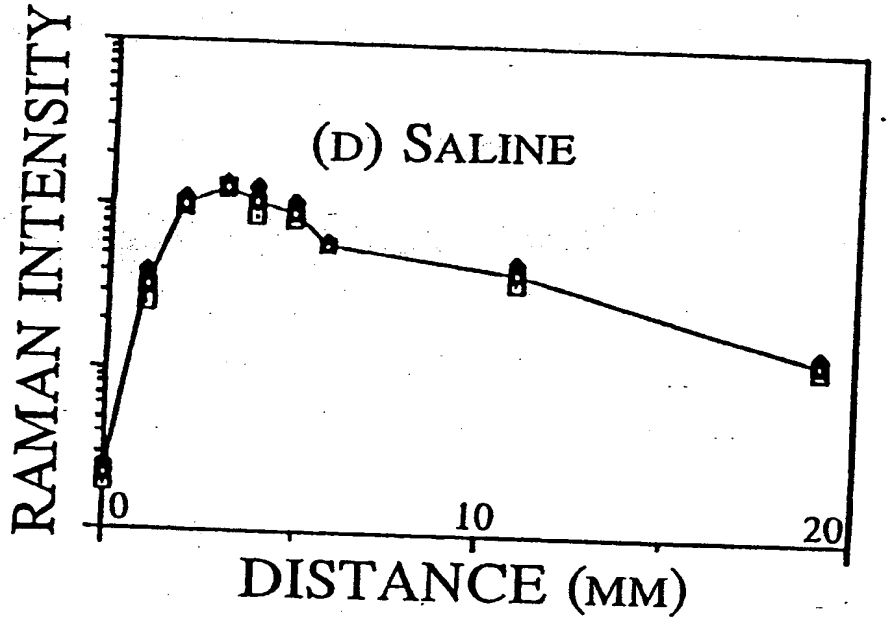

FIG. 13d

BNSDOCID: <WO___9303672A1_I_>

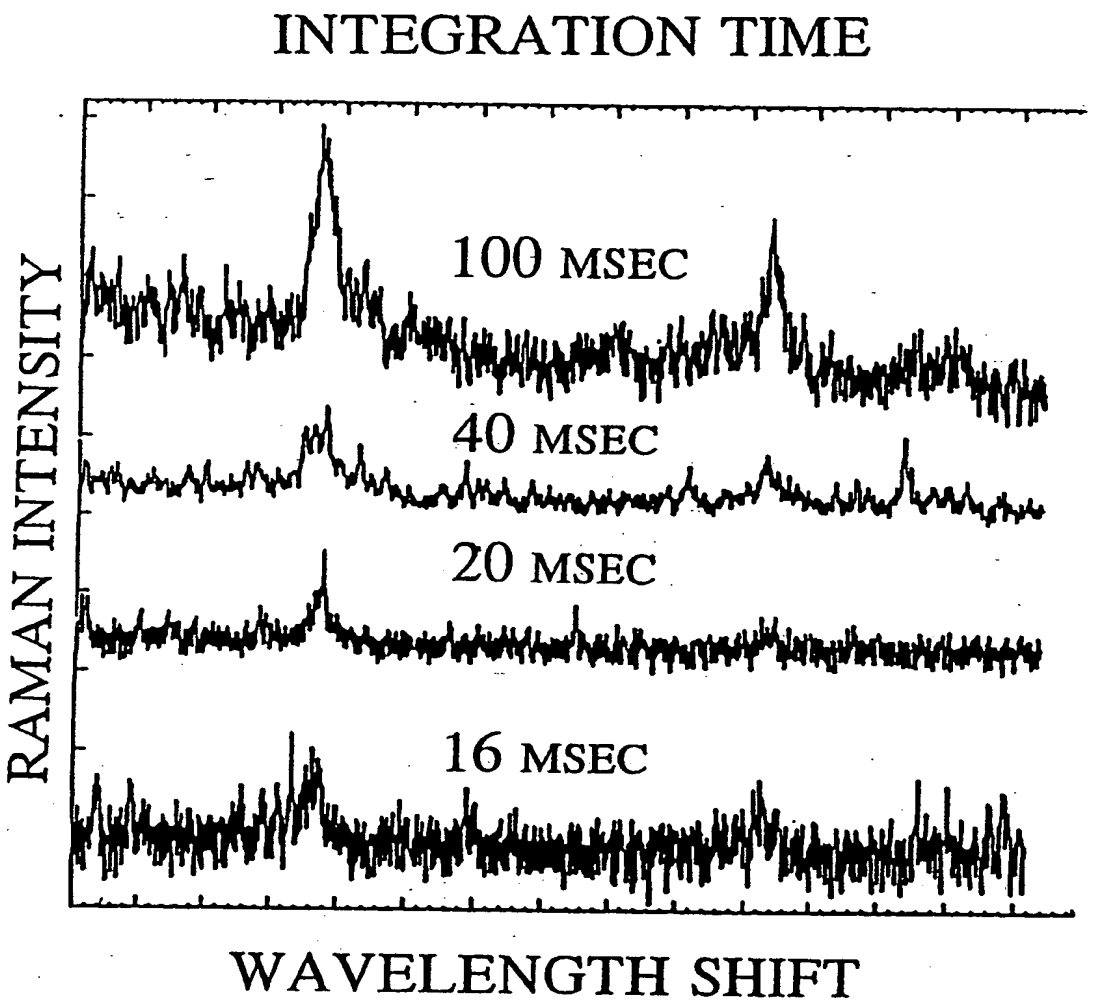

FIG. 14

BNSDOCID: <WO___9303672A1_I_>

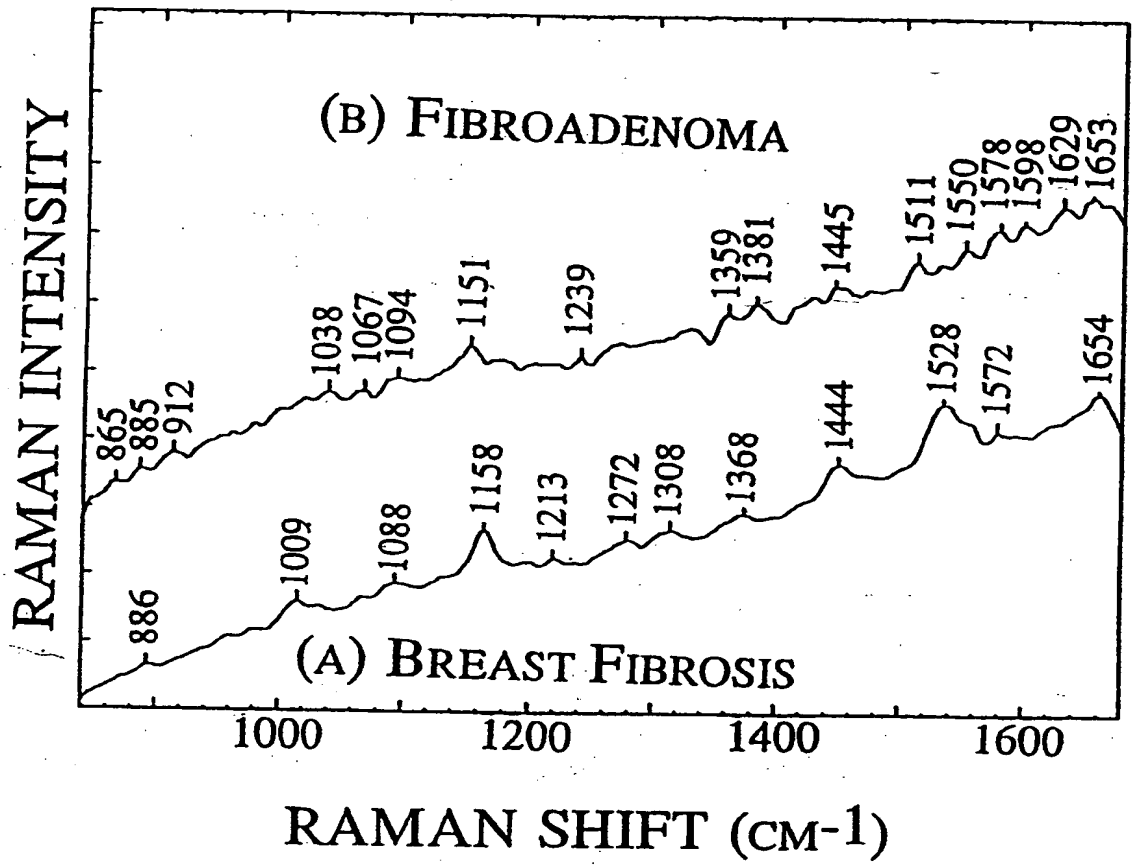

FIG. 15

BNSDOCID: <WO__9303672A1_I_>

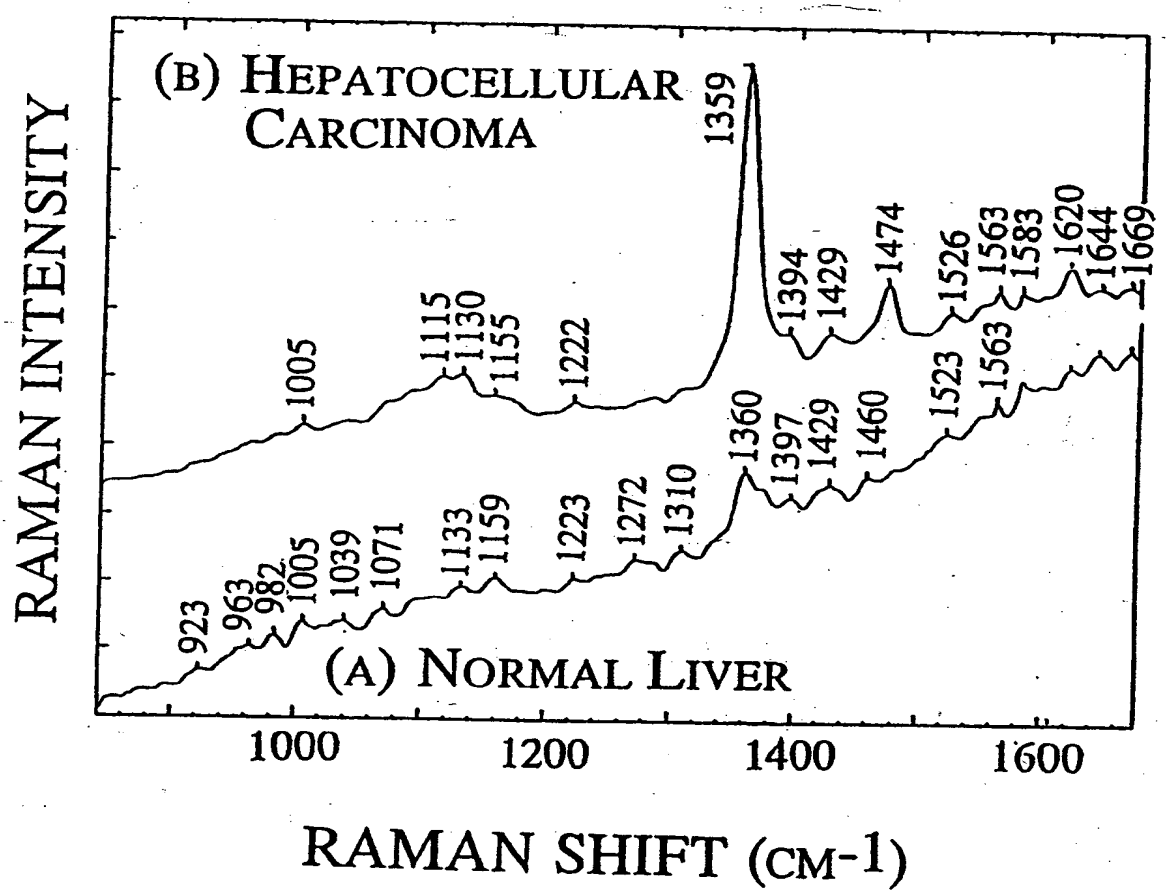

FIG. 16

BNSDOCID: <WO___9303672A1_I_>

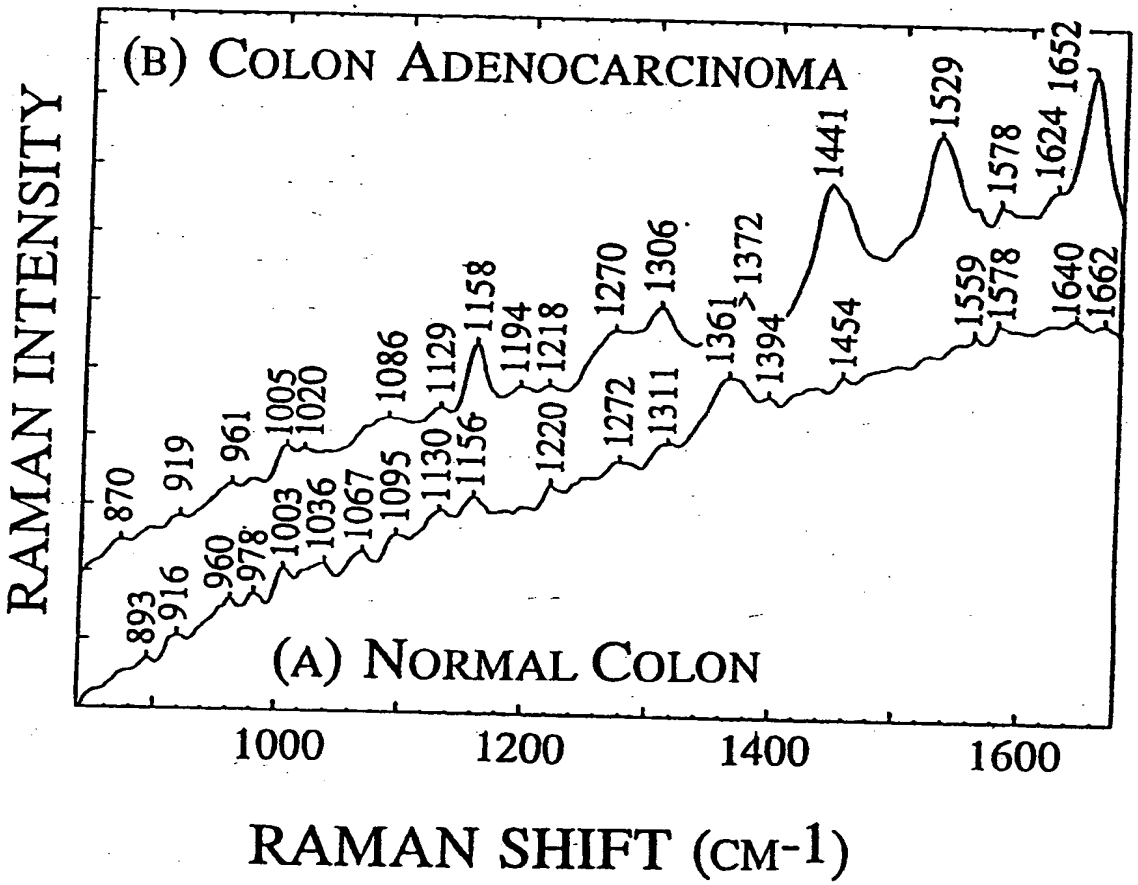

FIG. 17

BNSDOCID: <WO___9303672A1_I_>

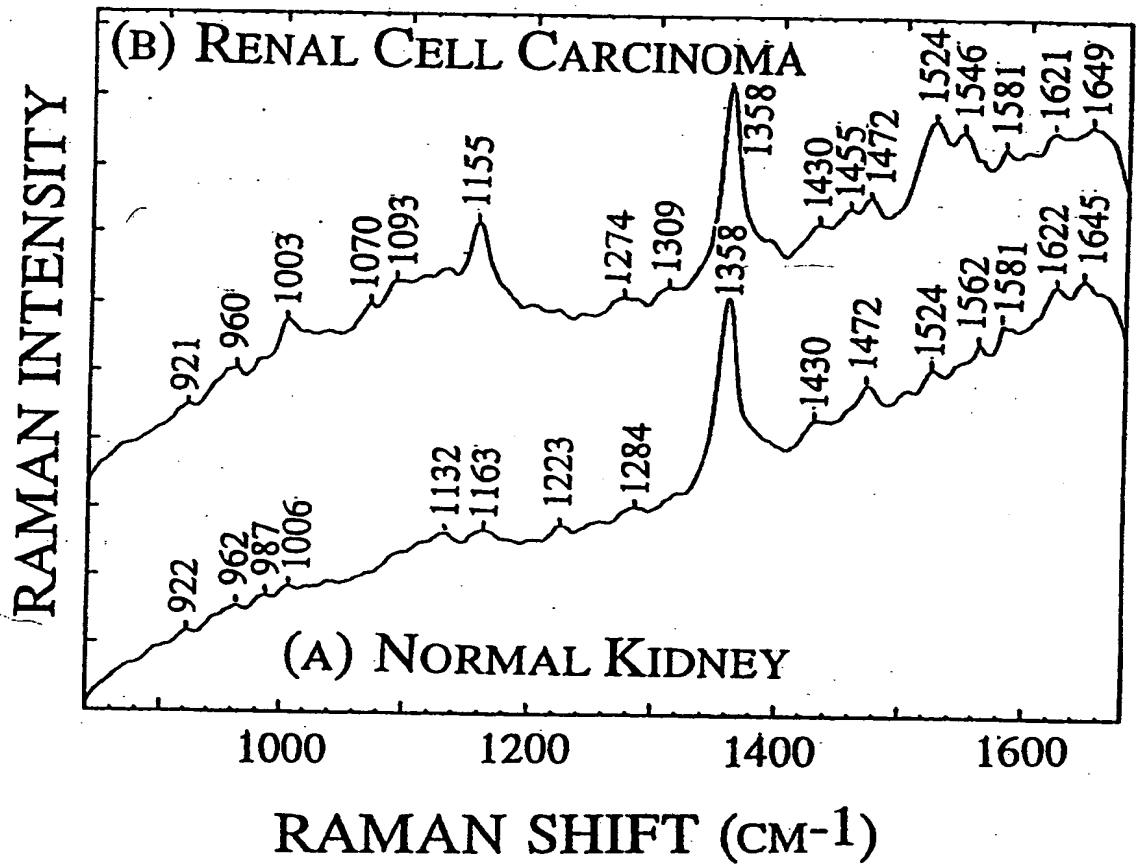

FIG. 18

BNSDOCID: <WO 9303672A1 I >

BREAST CARCINOMA

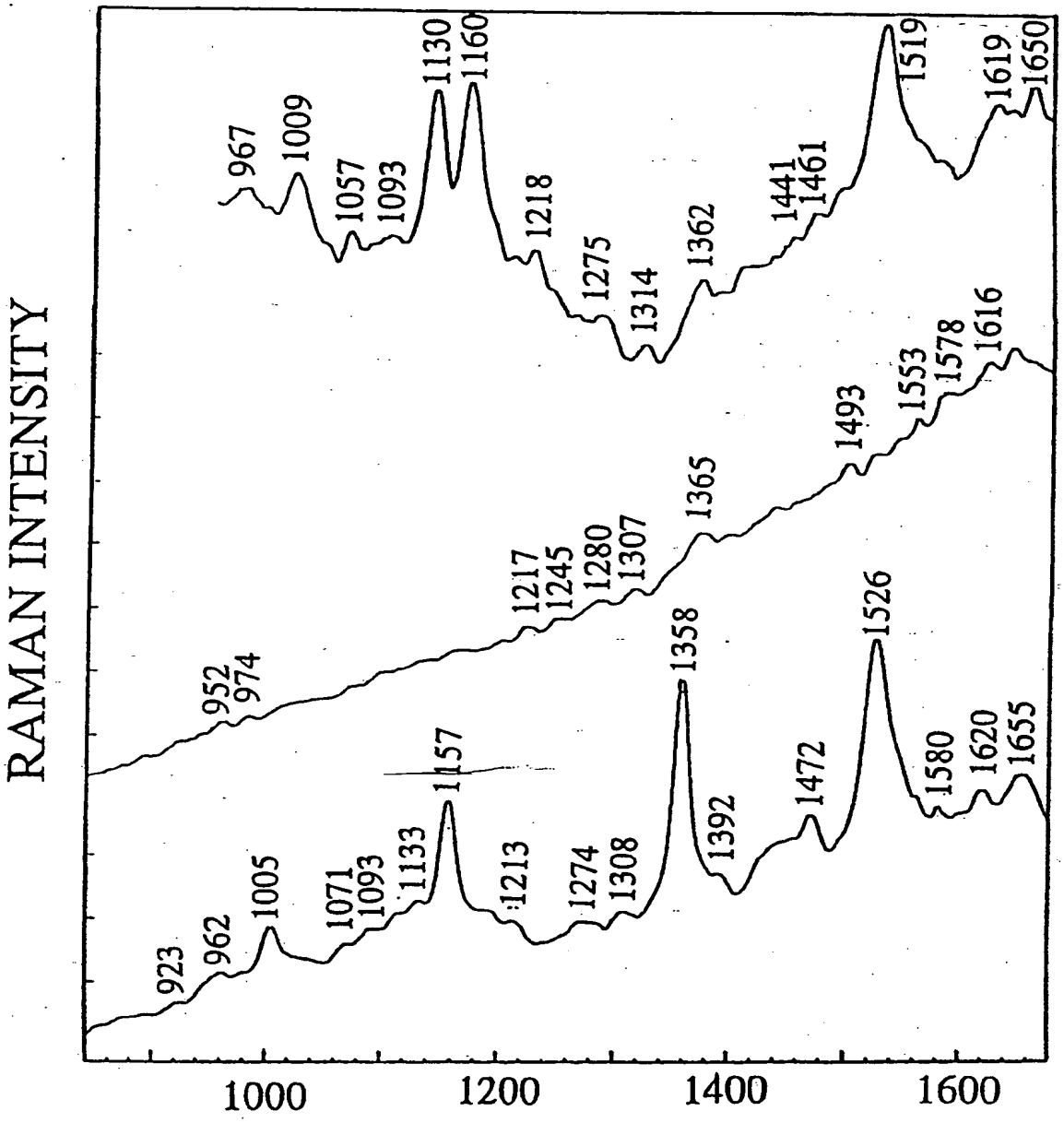

FIG. 19

BNSDOCID: <WO 9303672A1 I >

INTERNATIONAL SEARCH REPORT

PCT/US92/07040

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61B 5/06
US CL :606/7

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 128/632-634,665,666; 606/2,3,13-19

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US, A, 4,669,467 (WILLET ET AL.) 02 June 1987, See the entire document.	1,4,8-10, 13-24,28-35,41-43 2,3,6,7,11, 12,25-27,36-40,44,45
Y	Circulation; August 1988; PRINCE ET AL., "Increased Preferential Absorption in Human Atherosclerotic Plaque with Oral Beta Carotene, pp. 338-344, See the entire document.	2,3,6,7, 11,12,25-27,36-40, 44,45

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 DECEMBER 1992	Date of mailing of the international search report 12 JAN 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer Nguyen Ngoc-Ho DAVID SHAY NGUYEN NGOC-HO INTERNATIONAL DIVISION Telephone No. (703) 308-0858

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BNSDOCID: <WO_ 9303672A1 I >